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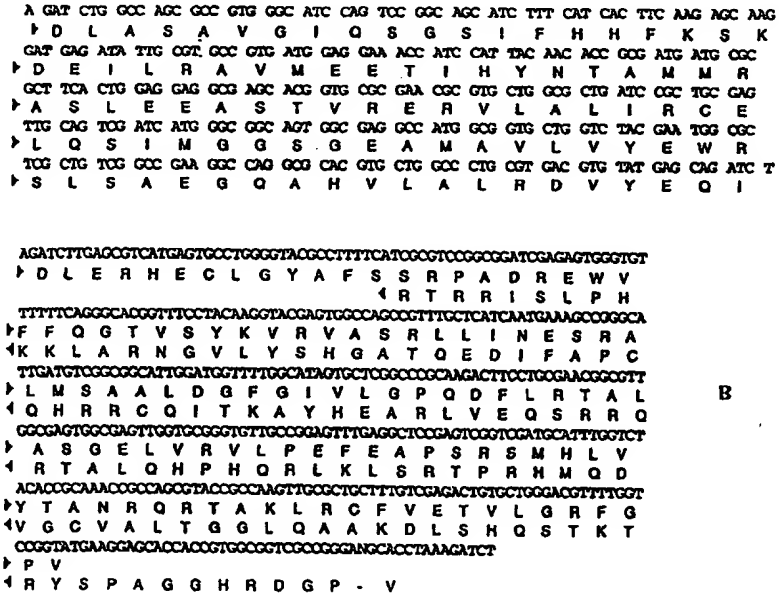

(57) Abstract

The present invention provides nucleic acid and amino acid sequence of the novel I–1 and I–2 polypeptides, which are associated with human inflammatory bowel disease (IBD). Methods of diagnosing and treating inflammatory bowel disease using the IBD–associated I–1 and I–2 antigens also are provided.

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IBD-ASSOCIATED MICROBIAL ANTIGENS AND METHODS OF USING SAME

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BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The invention relates generally to the fields of immunology, microbiology and inflammatory bowel disease and more specifically to the diagnosis and treatment of inflammatory bowel disease using microbial antigens.

BACKGROUND INFORMATION

Inflammatory bowel disease (IBD) is the collective term used to describe two gastrointestinal disorders of unknown etiology: Crohn's disease (CD) and ulcerative colitis (UC). The course and prognosis of IBD, which occurs world-wide and is reported to afflict as many as two million people, varies widely. Onset of IBD is predominantly in young adulthood with diarrhea, abdominal pain, and fever the three most common presenting symptoms. The diarrhea may range from mild to severe, and anemia and weight loss are additional common signs of IBD. Ten percent to fifteen percent of all patients with IBD will require surgery over a ten year period. In addition, patients with IBD are at increased risk for the development of intestinal cancer. Reports of an increased occurrence of psychological problems,

including anxiety and depression, are perhaps not surprising symptoms of what is often a debilitating disease that strikes people in the prime of life.

Unfortunately, the available therapies for inflammatory bowel disease are few, and both diagnosis and treatment have been hampered by a lack of knowledge regarding the etiology of the disease. What is clear, however, is that a combination of genetic factors, exogenous triggers and endogenous microflora can contribute to the immune-mediated damage to the intestinal mucosa seen in inflammatory bowel disease. In Crohn's disease, bacteria have been implicated in initiation and progression of the disease: the intestinal inflammation in Crohn's disease is notable for its frequent responsiveness to antibiotics and susceptibility to bacterial fecal flow. Common intestinal colonists and novel pathogens have been implicated in Crohn's by direct detection or by disease associated anti-microbial immune responses. Furthermore, in many genetically susceptible animal models of chronic colitis, lumenal micro-organisms are a necessary cofactor for disease; animals housed in a germ-free environment do not develop colitis. However, despite much direct and indirect evidence for a role for enteric microorganisms in Crohn's disease, the pathogenic organisms and antigens contributing to the immune dysregulation seen in this disease have not been identified.

Current diagnostic assays for Crohn's disease are unable to detect all patients with the disease. Thus, identification of novel microbial antigens associated with Crohn's disease would provide reagents that can increase the sensitivity of current diagnostic assays. In addition, such microbial antigens can bear a

disease related T-cell epitope and, as original or contributing inducers of the disease-related immune response, can be effective tolerogenic antigens for treating inflammatory bowel disease. Identification of IBD-associated microbial antigens also would facilitate isolation of the involved microbial species, paving the way for the discovery of new antibiotics or drugs for treating inflammatory bowel disease, such drugs ameliorating disease by eliminating the microbial inducers of disease.

Thus, there is a need for identification and isolation of microbial IBD-associated antigens for diagnosing and treating the many individuals suffering from inflammatory bowel disease. The present invention satisfies this need by providing the IBD-associated I-1 and I-2 microbial antigens. Related advantages are provided as well.

SUMMARY OF THE INVENTION

The present invention provides an isolated inflammatory bowel disease-associated I-2 polypeptide having substantially the same amino acid sequence as SEQ ID NO: 2. The invention also provides an isolated immunoreactive fragment of an I-2 polypeptide having substantially the same amino acid sequence as a portion of SEQ ID NO: 2. An isolated immunoreactive fragment of an I-2 polypeptide can have, for example, at least ten contiguous amino acids of SEQ ID NO: 2.

Also provided by the present invention is substantially purified antibody material that selectively binds an I-2 polypeptide having SEQ ID NO: 2. Such a substantially purified antibody material can be, for

example, substantially purified polyclonal or monoclonal antibody material.

The invention further provides an isolated nucleic acid molecule having a nucleic acid sequence encoding substantially the same amino acid sequence as SEQ ID NO: 2. An isolated nucleic acid molecule of the invention can have, for example, the nucleic acid sequence SEQ ID NO: 1.

Also provided by the invention is a method of diagnosing inflammatory bowel disease (IBD) in a subject. The method includes the steps of obtaining a sample from the subject; contacting the sample with an I-2 polypeptide, or immunoreactive fragment thereof, under conditions suitable to form a complex of the I-2 polypeptide, or the immunoreactive fragment thereof, and antibody to the I-2 polypeptide; and detecting the presence or absence of the complex, where the presence of the complex indicates that the subject has IBD. A method of the invention for diagnosing inflammatory bowel disease can be useful, for example, for diagnosing Crohn's disease. In a method of the invention for diagnosing inflammatory bowel disease, the presence or absence of the complex can be detected, for example, with a detectable secondary antibody that has specificity for a class determining portion of the antibody to the I-2 polypeptide.

Further provided by the invention is a method of inducing tolerance in a patient with inflammatory bowel disease by administering an effective dose of an I-2 polypeptide, or tolerogenic fragment thereof, to the patient with IBD. The methods of the invention can be particularly useful for treating a patient having Crohn's

disease. In a method of the invention for inducing tolerance, the I-2 polypeptide to be administered can have, for example, the amino acid sequence of SEQ ID NO: 2.

The invention also provides a composition including an I-2 polypeptide having substantially the same amino acid sequence as SEQ ID NO: 2, or tolerogenic fragment thereof, combined with a tolerogizing molecule. In a composition of the invention, the I-2 polypeptide can have, for example, the amino acid sequence SEQ ID NO: 2. A tolerogenic fragment useful in a composition of the invention can have, for example, at least ten contiguous amino acids of SEQ ID NO: 2.

The present invention also provides a method of identifying an agent useful in treating inflammatory bowel disease. The method includes the steps of obtaining a specimen of an enteric microbe from a patient with inflammatory bowel disease; isolating from the specimen a microbial species that includes a nucleic acid molecule encoding an I-2 polypeptide; contacting the microbial species with an agent; and assaying for reduced growth or viability of the microbial species as compared to the growth or viability in the absence of the agent, where the reduced growth or viability of the microbial species indicates that the agent is an agent useful in treating inflammatory bowel disease. A method of the invention can be useful, for example, for identifying an agent for treating Crohn's disease. The methods of the invention can be particularly useful for screening agents which are antibiotics.

The invention additionally provides a method of identifying an agent useful in treating inflammatory bowel disease using a novel animal model. The method includes the steps of administering an I-2 polypeptide to a non-human animal, whereby one or more symptoms of IBD are exhibited; administering an agent to the non-human animal; and assaying the level of the one or more symptoms characteristic of IBD, where a reduction in the level of the one or more symptoms as compared to a control level indicates that the agent is an agent useful in treating IBD. The methods of the invention can be applied, for example, to identification of agents useful in treating Crohn's disease. The I-2 polypeptide administered can have, for example, the amino acid sequence SEQ ID NO: 2. A non-human animal particularly useful in the methods of the invention can be, for example, a mouse deficient in Gαi2, TCRα or IL-10.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the I-1 and I-2 nucleic acid sequences and encoded polypeptides. A. Shown are the I-2 nucleic acid sequence (SEQ ID NO: 1) and predicted amino acid sequence (SEQ ID NO: 2). B. Shown are the I-1 nucleic acid sequence (SEQ ID NO: 3) and I-1 open reading frame 1 (top; SEQ ID NO: 4) and open reading frame 2 (bottom; SEQ ID NO: 5).

Figure 2 shows an alignment of the I-2 amino acid sequence SEQ ID NO: 2 with homologous sequences. Sequences were identified by tBLASTx search against the non-redundant database. Alignments were generated by CLUSTALW, with modifications by Genedoc. "Clostridium" is the predicted protein 4 from *Clostridium pasteurianum* (SEQ ID NO: 6; accession number 481591); "Mycobacterium"

is the predicted protein Rv3557c from *Mycobacterium tuberculosis* (SEQ ID NO: 7; accession number 1877288); and "Aquifex" is a transcriptional regulator from *Aquifex aeolicus* (SEQ ID NO: 8; accession number 2984362). Residues identical or conservatively substituted among four polypeptides are shown in black; residues identical or conservatively substituted among three polypeptides are shown in dark gray; and residues identical or conservatively substituted between two polypeptides are shown in light gray. Consensus residues are shown beneath the alignment, with one-letter codes indicating conserved amino acids; "6" indicating a conserved non-polar (hydrophobic) residue such as leucine, isoleucine, alanine, valine or methionine; and "5" indicating the conserved aromatic residue phenylalanine or tyrosine.

Figure 3 shows the results of PCR analysis with I-2 (SEQ ID NO: 1) specific primers using paraffin embedded colonic samples from CD patients, UC patients and control individuals. "N" designates the number of samples assayed, and "N+" designates the number of positive samples. "Ca" designates cancer resection samples; "Divertic" designates diverticulitis samples; "append" designates appendicitis samples; and "I-col " designates ischemic colitis.

Figure 4 shows IgG and IgA reactivities to the I-2 polypeptide (SEQ ID NO: 2) in various populations. A. Sera from Crohn's disease patients and normal individuals was assayed by ELISA at the indicated dilutions for IgG reactivity to the GST-I-2 fusion polypeptide (SEQ ID NO: 2). The absorbance values were calculated by subtracting absorbance to GST alone.

B. Sera from Crohn's disease, ulcerative colitis and normal individuals was assayed by ELISA for IgA reactivity to the GST-I-2 fusion polypeptide (SEQ ID NO: 2). The absorbance values were calculated by subtracting absorbance to GST alone. The cut-off (dotted line) was set as two standard deviations above the mean value for the normal population.

Figure 5 shows T cell proliferation assays using $CD4^+$ T cells derived from normal C57BL/6J mice. T cells were stimulated with the indicated concentration of GST-I-2 fusion protein, shown on the Y axis. The X axis represents counts of incorporated [^{3}H]-thymidine. SEB represents T cells stimulated with the positive control *Staphylococcus aureus* enterotoxin B.

Figure 6 shows T cell cytokine expression in response to challenge with the GST-I-2 fusion protein.

Figure 7 shows a variety of conditions for culturing microbial organisms isolated from IBD patients.

Figure 8 shows activation of naive $CD4^+$ T cells upon I-2 stimulation. (A) [^{3}H]-thymidine proliferation assay of native $CD4^+$ T cells. (B) [^{3}H]-thymidine proliferation assay of memory $CD4^+$ T cells.

Figure 9 shows that recombinant I-2 is a superantigen. (A) Splenic C57 BL/6J $CD4^+$ T cells were cultured with antigen-presenting cells from C57 BL/6 that were pulsed with I-2-GST, or fixed with paraformadehyde and then pulsed with I-2-GST overnight. (B) Pigeon cytochrome C (PPC) specific $CD4^+$ T cells were cultured with syngenic antigen-presenting cells pulsed with PPC, or fixed with paraformadehyde and then pulsed with PPC.

DETAILED DESCRIPTION OF THE INVENTION

The pathogenesis of inflammatory bowel disease, although poorly understood, ultimately involves immune-mediated tissue damage. Similar to autoimmune disorders such as diabetes mellitus and multiple sclerosis, inflammatory bowel disease is associated with various immunologic abnormalities and can represent a process of immune dysfunction. However, unlike the other disorders, inflammatory bowel disease occurs in a mucosal site interfacing with the intestinal lumen, and, therefore, a primary immune target in inflammatory bowel disease can be extrinsic agent such as a chronic microbial colonist. In this case, the mucosal injury characteristic of inflammatory bowel disease is a consequence of inflammatory bystander damage to resident parenchymal cells.

The present invention is directed to the exciting discovery that several microbial DNA sequences are found preferentially in involved Crohn's disease (CD) mucosa as compared to uninvolved mucosa. As disclosed herein, representational difference analysis (RDA), a PCR driven subtractive cloning approach for identifying DNA sequences found preferentially in an infected area, was used to isolate DNA sequences from a Crohn's disease patient that were differentially present in mononuclear cells from the lamina propria in an area with ulcerations as compared to an area macroscopically free of disease (see Example I). As disclosed herein, two IBD-associated sequences were of microbial origin. The nucleic acid and amino acid sequences, designated I-1 (SEQ ID NOS: 3 to 5) and I-2 (SEQ ID NOS: 1 and 2) are shown in Figure 1. As further disclosed herein, PCR analysis of colonic samples from CD, UC and non-IBD patients revealed that the I-2

sequence (SEQ ID NO: 1) was more often found in involved CD tissue than in UC or non-IBD samples (Example I and Figure 3). Thus, novel microbial sequences have been identified that are associated with inflammatory bowel disease, in particular, with inflamed CD lesions. Isolation of microbial sequences associated with IBD implicates microbes in the pathogenesis of IBD and provides valuable reagents for diagnosing or ameliorating inflammatory bowel disease.

Thus, the present invention provides an isolated inflammatory bowel disease associated I-2 polypeptide having substantially the same amino acid sequence as SEQ ID NO: 2. The invention also provides an isolated immunoreactive fragment of an I-2 polypeptide having substantially the same amino acid sequence as a portion of SEQ ID NO: 2. An isolated immunoreactive fragment of an I-2 polypeptide can have, for example, at least ten contiguous amino acids of SEQ ID NO: 2.

The term "isolated," as used herein in reference to a polypeptide means a polypeptide that is in a form that is relatively free from contaminating lipids, polypeptides, nucleic acids or other cellular material normally associated with the polypeptide in a cell.

As used herein, the term "I-2 polypeptide" means a polypeptide having substantially the same amino acid sequence as the microbial I-2 polypeptide (SEQ ID NO: 2) shown in Figure 1A. The microbial I-2 polypeptide (SEQ ID NO: 2) is a polypeptide of 100 amino acids sharing some similarity to bacterial transcriptional regulators, with the greatest similarity in the amino-terminal 30 amino acids. As illustrated in Figure 2, the I-2 polypeptide shares weak homology with

the predicted protein 4 from *C. pasteurianum* (SEQ ID NO: 6); Rv3557c from *Mycobacterium tuberculosis* (SEQ ID NO: 7); and a transcriptional regulator from *Aquifex aeolicus* (SEQ ID NO: 8). As disclosed in Example I, the I-2 encoding nucleic acid (SEQ ID NO: 1) is differentially present in involved Crohn's disease tissue, as compared to mucosa macroscopically free of disease.

An I-2 polypeptide having substantially the same amino acid sequence as SEQ ID NO: 2 can be the naturally occurring I-2 polypeptide (SEQ ID NO: 2) or a related polypeptide having substantial amino acid sequence similarity to this sequence. Such related polypeptides exhibit greater sequence similarity to the I-2 polypeptide SEQ ID NO: 2 than to the *C. pasteurianum* sequence SEQ ID NO: 6 and include isotype variants or homologs of the amino acid sequence shown in Figure 1A. As used herein, the term I-2 polypeptide generally describes polypeptides generally having an amino acid sequence with greater than about 50% identity, preferably greater than about 60% identity, more preferably greater than about 70% identity, and can be a polypeptide having greater than about 80%, 90%, 95%, 97%, or 99% amino acid sequence identity with SEQ ID NO: 2, said amino acid identity determined with CLUSTALW using the BLOSUM 62 matrix with default parameters. The *C. pasteurianum* protein 4 (SEQ ID NO: 6) has about 21% amino acid identity with the I-2 polypeptide SEQ ID NO: 2 and, therefore, is not an I-2 polypeptide as defined herein.

The I-1 nucleic acid sequence (SEQ ID NO: 3) contains two open reading frames (ORFs) on opposite strands. The top strand I-1 ORF shown in Figure 1B encodes a predicted polypeptide of 115 amino acids (SEQ

ID NO: 4) with homology to the prokaryotic transcription factor ptxR. The bottom strand ORF encodes a predicted polypeptide of 114 amino acids (SEQ ID NO: 5). The I-1 encoding nucleic acid (SEQ ID NO: 3) also was isolated by RDA analysis as a sequence preferentially found in involved CD mucosa (see Example I).

An I-1 polypeptide can have substantially the same amino acid sequence as SEQ ID NO: 4 or SEQ ID NO: 5. Such an I-1 polypeptide can be a naturally occurring I-1 polypeptide (SEQ ID NOS: 4 or 5) or a related polypeptide, for example, an isotype variant or homologous sequence from a different bacterial species having substantial amino acid sequence similarity to one of these sequences. As used herein, the term I-1 polypeptide generally describes polypeptides generally having an amino acid sequence with greater than about 50% identity, preferably greater than about 60% identity, more preferably greater than about 70% identity, and can be a polypeptide having greater than about 80%, 90%, 95%, 97%, or 99% amino acid sequence identity with SEQ ID NOS: 4 or 5, said amino acid identity determined with CLUSTALW using the BLOSUM 62 matrix with default parameters.

As used herein, the term "substantially the same amino acid sequence," when used in reference to an I-1 or I-2 polypeptide, is intended to mean a sequence as shown in Figure 1A or Figure 1B, or a similar, non-identical sequence that is considered by those skilled in the art to be a functionally equivalent amino acid sequence. For example, an amino acid sequence that has substantially the same amino acid sequence as an I-1 polypeptide (SEQ ID NOS: 4 or 5) or I-2 polypeptide (SEQ ID NO: 2) can have one or more modifications such as

amino acid additions, deletions or substitutions relative to the amino acid sequence of SEQ ID NO: 4 or 5, or SEQ ID NO: 2, respectively, provided that the modified polypeptide retains substantially at least one biological activity of I-1 or I-2 such as immunoreactivity or tolerogenic activity, described further below. Comparison for substantial similarity between amino acid sequences is usually performed with sequences between about 6 and 100 residues, preferably between about 10 and 100 residues and more preferably between about 25 and 35 residues.

Thus, it is understood that limited modifications can be made to an I-1 or I-2 polypeptide, or to an immunoreactive or tolerogenic fragment thereof, as described further below, without destroying its biological function. A modification of an I-1 or I-2 polypeptide that does not destroy immunoreactivity or a modification of an I-1 or I-2 polypeptide that does not destroy tolerogenic activity is encompassed within the meaning of the term I-1 polypeptide, or I-2 polypeptide, as used herein. A modification can be, for example, an addition, deletion, or substitution of one or more amino acid residues; substitution of a compound that mimics amino acid structure or function; or addition of chemical moieties such as amino or acetyl groups. The activity of a modified I-1 or I-2 polypeptide or fragment thereof can be assayed, for example, using one of the assays for immunoreactivity or tolerogenic activity disclosed herein (see below).

A particularly useful modification of a polypeptide of the invention, or fragment thereof, is a modification that confers, for example, increased stability. Incorporation of one or more D-amino acids is

a modification useful in increasing stability of a polypeptide or polypeptide fragment. Similarly, deletion or substitution of lysine can increase stability by protecting against degradation. For example, such a substitution can increase stability and, thus, bioavailability of one of the polypeptide antigens disclosed herein.

The I-2 polypeptide, and fragments thereof, can be useful to prepare substantially purified antibody material that selectively binds an I-2 polypeptide (SEQ ID NO: 2). The antibody material can be, for example, substantially purified polyclonal antiserum or monoclonal antibody material. The antibody material of the invention be useful, for example, in determining the presence and location of I-2 polypeptide within the mucosa of afflicted patients and in diagnosing inflammatory bowel disease. The substantially purified antibody material of the invention can be useful, for example, in an ELISA or immunohistopathological assay for diagnosing Crohn's disease.

As used herein, the term "antibody material" is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as polypeptide fragments of antibodies that retain a selective binding activity for an I-2 polypeptide of at least about 1×10^5 M^{-1}. One skilled in the art would know that anti-I-2 antibody fragments such as Fab, $F(ab')_2$ and Fv fragments can retain selective binding activity for an I-2 polypeptide and, thus, are included within the definition of an antibody. In addition, the term antibody material as used herein encompasses non-naturally occurring antibodies and fragments containing, at a minimum, one V_H and one V_L domain, such as chimeric antibodies, humanized

antibodies, single chain Fv fragments (scFv) that selectively bind an I-2 polypeptide. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, produced recombinantly or obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Borrebaeck (Ed.), Antibody Engineering (Second edition) New York: Oxford University Press (1995), which is incorporated herein by reference.

Antibody material "selective for" an I-2 polypeptide, or that "selectively binds" an I-2 polypeptide, binds with substantially higher affinity to that polypeptide than to an unrelated polypeptide. The substantially purified antibody material of the invention also can be specific for an I-2 polypeptide, whereby its binding affinity is significantly higher for an I-2 polypeptide than for related polypeptides such as SEQ ID NOS: 6 to 8.

Anti-I-2 antibody material can be prepared, for example, using an I-2 fusion protein or a synthetic peptide encoding a portion of the I-2 polypeptide (SEQ ID NO: 2) as an immunogen. One skilled in the art would know that purified I-2 polypeptide, which can be produced recombinantly, or fragments of I-2, including peptide portions of I-2 such as synthetic peptides, can be used as an immunogen. Non-immunogenic fragments or synthetic peptides of I-2 can be made immunogenic by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). In addition, various other carrier molecules and methods for coupling a hapten to a carrier molecule are well known in the art as described, for example, by Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor

Laboratory Press, 1988), which is incorporated herein by reference.

The term "substantially purified," as used herein in reference to antibody material, means that the antibody material is substantially devoid of polypeptides, nucleic acids and other cellular material which with an antibody is normally associated in a cell. The claimed antibody material that selectively binds an I-2 polypeptide (SEQ ID NO: 2) further is substantially devoid of antibody material of unrelated specificities, i.e. that does not selectively bind an I-2 polypeptide. The antibody material of the invention can be prepared in substantially purified form, for example, by I-2 affinity purification of polyclonal anti-I-2 antisera, by screening phage displayed antibodies against the I-2 polypeptide (SEQ ID NO: 2), or as monoclonal antibodies prepared from hybridomas.

As disclosed herein, the I-2 sequence is present in mice housed under conventional conditions and induces a dose-dependent proliferative response as well as IL-10 secretion (Example V). As further disclosed herein, both naive and memory $CD4^+$ T cell populations spontaneously proliferate and produce IL-10 in response to I-2 (see Figure 8). Furthermore, the I-2 response is dependent on MHC class II-mediated recognition but does not require antigen processing, as indicated by the ability of paraformaldehyde-fixed cells to induce an I-2 response (see Figure 9). These results demonstrate that I-2 is a novel superantigen and indicate that methods of blocking an I-2 superantigen response can prevent or ameliorate Crohn's disease.

Thus, the present invention provides a method of treating a patient with IBD by administering to the patient an effective dose of an agent that selectively binds an I-2 polypeptide having SEQ ID NO: 2. In a method of the invention, a useful agent can be, for example, substantially purified antibody material, such as polyclonal or monoclonal antibody material, that selectively binds to an I-2 polypeptide having SEQ ID NO: 2. The methods of the invention are useful in treating patients with IBD such as patients having Crohn's disease.

Further provided by the invention is a method of treating a patient with IBD by inhibiting a superantigen response induced by an I-2 polypeptide having SEQ ID NO: 2. Such a superantigen response can be, for example, T cell proliferation or IL-10 secretion. A method of the invention can be particularly useful for inhibiting a superantigen response in a patient with Crohn's disease.

The term superantigen response as used herein refers to the response of a T cell to a superantigen and generally is a proliferative response. Superantigens, which are among the most potent T-cell mitogens known, bind residues in the Vβ domain of the T-cell receptor and residues in class II MHC molecules outside of the antigen-binding cleft. Thus, a superantigen can act to cross-link a T cell to a class II MHC molecule in an antigen-independent manner and activate all T cells expressing T-cell receptors with a particular Vβ domain.

The term inhibiting, as used herein in reference to a superantigen response, refers to a partial or complete reduction of a specific response induced by

the I-2 superantigen having SEQ ID NO: 2. As disclosed herein in Example V, the I-2 superantigen results in a proliferative response as well as IL-10 secretion. Thus, a partial or complete reduction in the proliferative response or IL-10 secretion can be used to identify an agent useful in a method of the invention.

The invention further provides an isolated nucleic acid molecule having a nucleic acid sequence encoding substantially the same amino acid sequence as SEQ ID NO: 2. An isolated nucleic acid molecule of the invention can have, for example, the nucleic acid sequence SEQ ID NO: 1. These nucleic acid molecules are useful, for example, in producing recombinant polypeptides and as probes for detecting I-2 mRNA expression. Nucleotide portions of SEQ ID NO: 1 also are useful, for example, as primers for PCR analysis (see Example I).

Isolated nucleic acid molecules of the invention include, for example, nucleic acid molecules encoding I-2 polypeptide homologs, nucleic acid molecules that are related, but different and encode the polypeptide of SEQ ID NO: 2 due to the degeneracy of the genetic code, and nucleic acid molecules that are related, but different and encode an I-2 polypeptide different from SEQ ID NO: 2 that exhibits immunoreactivity or tolerogenic activity.

The methods of the invention relate to the diagnosis and treatment of inflammatory bowel disease, which is a designation that encompasses the broad categories of Crohn's disease and ulcerative colitis. Crohn's disease (regional enteritis) is a disease of chronic inflammation that can involve any part of the

gastrointestinal tract. Commonly the distal portion of the small intestine (ileum) and cecum are affected. In other cases, the disease is confined to the small intestine, colon or anorectal region. Crohn's disease occasionally involves the duodenum and stomach, and more rarely the esophagus and oral cavity.

The variable clinical manifestations of Crohn's disease are, in part, a result of the varying anatomic localization of the disease. The most frequent symptoms of CD are abdominal pain, diarrhea and recurrent fever. CD is commonly associated with intestinal obstruction or fistula, which is an abnormal passage between diseased loops of bowel, for example. Crohn's disease also includes complications such as inflammation of the eye, joints and skin; liver disease; kidney stones or amyloidosis. In addition, CD is associated with an increased risk of intestinal cancer.

Several features are characteristic of the pathology of Crohn's disease. The inflammation associated with CD, known as transmural inflammation, involves all layers of the bowel wall. Thickening and edema, for example, typically also appear throughout the bowel wall, with fibrosis also present in long-standing disease. The inflammation characteristic of CD also is discontinuous in that segments of inflamed tissue, known as "skip lesions," are separated by apparently normal intestine. Furthermore, linear ulcerations, edema, and inflammation of the intervening tissue lead to a "cobblestone" appearance of the intestinal mucosa, which is distinctive of CD.

A hallmark of Crohn's disease is the presence of discrete aggregations of inflammatory cells, known as

granulomas, which are generally found in the submucosa. Some Crohn's disease cases display the typical discrete granulomas, while others show a diffuse granulomatous reaction or nonspecific transmural inflammation. As a result, the presence of discrete granulomas is indicative of CD, although the absence of granulomas also is consistent with the disease. Thus, transmural or discontinuous inflammation, rather than the presence of granulomas, is a preferred diagnostic indicator of Crohn's disease (Rubin and Farber, Pathology (Second Edition) Philadelphia: J.B. Lippincott Company (1994), which is incorporated herein by reference).

Ulcerative colitis (UC) is a disease of the large intestine characterized by chronic diarrhea with cramping abdominal pain, rectal bleeding, and loose discharges of blood, pus and mucus. The manifestations of ulcerative colitis vary widely. A pattern of exacerbations and remissions typifies the clinical course of most UC patients (70%), although continuous symptoms without remission are present in some patients with UC. Local and systemic complications of UC include arthritis, eye inflammation such as uveitis, skin ulcers and liver disease. In addition, ulcerative colitis and especially long-standing, extensive disease is associated with an increased risk of colon carcinoma.

Several pathologic features characterize UC in distinction to other inflammatory bowel diseases. Ulcerative colitis is a diffuse disease that usually extends from the most distal part of the rectum for a variable distance proximally. The term left-sided colitis describes an inflammation that involves the distal portion of the colon, extending as far as the splenic flexure. Sparing of the rectum or involvement of

the right side (proximal portion) of the colon alone is unusual in ulcerative colitis. The inflammatory process of ulcerative colitis is limited to the colon and does not involve, for example, the small intestine, stomach or esophagus. In addition, ulcerative colitis is distinguished by a superficial inflammation of the mucosa that generally spares the deeper layers of the bowel wall. Crypt abscesses, in which degenerated intestinal crypts are filled with neutrophils, also are typical of ulcerative colitis (Rubin and Farber, *supra*, 1994).

In comparison with Crohn's disease, which is a patchy disease with frequent sparing of the rectum, ulcerative colitis is characterized by a continuous inflammation of the colon that usually is more severe distally than proximally. The inflammation in ulcerative colitis is superficial in that it is usually limited to the mucosal layer and is characterized by an acute inflammatory infiltrate with neutrophils and crypt abscesses. In contrast, Crohn's disease affects the entire thickness of the bowel wall with granulomas often, although not always, present. Disease that terminates at the ileocecal valve, or in the colon distal to it, is indicative of ulcerative colitis, while involvement of the terminal ileum, a cobblestone-like appearance, discrete ulcers or fistulas suggest Crohn's disease. Characteristics that serve to distinguish Crohn's disease from ulcerative colitis are summarized in Table 1 (Rubin and Farber, *supra*, 1994).

Table 1

Characteristic Features of Crohn's disease and ulcerative colitis

Feature	Crohn's Disease	Ulcerative Colitis
Macroscopic		
Thickened bowel wall	Typical	Uncommon
Luminal narrowing	Typical	Uncommon
"Skip" lesions	Common	Absent
Right colon predominance	Typical	Absent
Fissures and fistulas	Common	Absent
Circumscribed ulcers	Common	Absent
Confluent linear ulcers	Common	Absent
Pseudopolyps	Absent	Common
Microscopic		
Transmural inflammation	Typical	Uncommon
Submucosal fibrosis	Typical	Absent
Fissures	Typical	Rare
Granulomas	Common	Absent
Crypt abscesses	Uncommon	Typical

Certain immune-mediated disorders, including systemic lupus erythematosis, primary biliary cirrhosis and autoimmune hepatitis, are closely associated with distinctive patterns of autoantibody production. Disease-specific marker antibodies also have been observed in IBD. In the case of ulcerative colitis, anti-neutrophil cytoplasmic antibodies that produce a perinuclear staining pattern (pANCA), for example, upon indirect immunofluorescence microscopy of alcohol-fixed neutrophils, are elevated in 68-80% of UC patients and less frequently in other disorders of the colon. In

Crohn's disease, serum reactivity to the cell wall mannan polysaccharide of *Saccharomyces uvarum* (brewer's yeast) is a serologic marker for a majority of individuals with Crohn's disease (Sendid et al., Clin. Diag. Lab. Immunol., 3:219-226 (1996), which is incorporated herein by reference).

As disclosed herein, ELISA analysis showed increased IgG serum reactivity to a GST-I-2 fusion polypeptide (SEQ ID NO: 2) in patients with Crohn's disease as compared to normal individuals (see Figure 4A). As shown in Figure 4B, 9 of 10 Crohn's disease patients had IgA serum reactivity to the GST-I-2 fusion polypeptide greater than two standard deviations above the mean value for a normal population. In contrast, no normal serum samples contained anti-I-2 IgA reactivity above this cutoff. The anti-I-2 serum IgA reactivity also was significantly higher on average in samples from Crohn's disease patients as compared to ulcerative colitis patients. These results indicate that reactivity to the I-2 polypeptide (SEQ ID NO: 2) can be used to differentiate Crohn's disease from normal individuals and those with UC.

Based on the above findings, the present invention provides methods of diagnosing inflammatory bowel disease (IBD) in a subject. The methods include the steps of obtaining a sample from the subject; contacting the sample with an I-2 polypeptide, or immunoreactive fragment thereof, under conditions suitable to form a complex of the I-2 polypeptide, or the immunoreactive fragment thereof, and antibody to the I-2 polypeptide; and detecting the presence or absence of the complex, where the presence of the complex indicates that the subject has IBD. A method of the invention for

diagnosing inflammatory bowel disease can be useful, for example, for diagnosing Crohn's disease. The presence or absence of the complex can be detected, for example, with a detectable secondary antibody that has specificity for a class determining portion of the antibody to the I-2 polypeptide.

As used herein, the term "subject" means any animal capable of having inflammatory bowel disease, including a human, non-human primate, rabbit, rat or mouse, especially a human. A subject generally has one or more symptoms of ulcerative colitis or Crohn's disease.

A sample useful in the methods of the invention can be obtained from any biological fluid having antibodies such as, for example, whole blood, plasma, saliva, or other bodily fluid or tissue, preferably serum. A sample to be assayed according to the methods of the invention can be obtained from any such subject.

As used herein, the term "complex" is synonymous with "immune complex" and means an aggregate of two or more molecules that results from specific binding between an antigen, such as a protein or peptide, and an antibody. In the methods of the invention, a complex is formed by specific binding of an I-2 polypeptide to an antibody.

In the methods of the invention, a complex can be detected with a detectable secondary antibody that has specificity for a class determining portion of the antibody to the I-2 polypeptide. Such a secondary antibody can be, for example, an anti-IgA secondary antibody, an anti-IgG secondary antibody, or a

combination of anti-IgA and anti-IgG secondary antibodies.

As used herein, the term "secondary antibody" means an antibody or combination of antibodies, which binds an antibody that specifically binds an I-2 polypeptide having substantially the amino acid sequence SEQ ID NO: 2. One skilled in the art understands that, preferably, a secondary antibody does not compete with the I-2 antigen for binding to the primary antibody. A secondary antibody can bind any epitope of the antibody that specifically binds the I-2 polypeptide. A particularly useful secondary antibody is an anti-IgA or anti-IgG antibody having specificity for the class determining portion of the primary antibody. A useful secondary antibody is specific for the species from which the sample was obtained. For example, if human serum is the sample to be assayed, mouse anti-human IgA or IgG can be a useful secondary antibody. A combination of different antibodies, which can be useful in the methods of the invention, also is encompassed within the meaning of the term secondary antibody, provided that at least one antibody of the combination reacts with an antibody that specifically binds an I-2 polypeptide.

As used herein, the term "class determining portion," when used in reference to a secondary antibody, means the heavy chain constant-region sequence of an antibody that determines the isotype, such as IgA, IgD, IgE, IgG or IgM. Thus, a secondary antibody that has specificity for the class determining portion of an IgA molecule, for example, binds IgA in preference to other antibody isotypes.

A secondary antibody useful in the invention can be obtained commercially or by techniques well known in the art. Such an antibody can be a polyclonal or, preferably, monoclonal antibody. For example, IgG reactive polyclonal antibodies can be prepared using IgG or Fc fragments of IgG as an immunogen to stimulate the production of antibodies in the antisera of an animal such as a rabbit, goat, sheep or rodent, as described in Harlow and Lane, Antibodies: A Laboratory Manual New York: Cold Spring Harbor Laboratory (1988), which is incorporated herein by reference. Monoclonal secondary antibodies, which are a population of antibody molecules that contain only one species of idiotope capable of binding a particular antigen epitope also can be produced by routine methods (see, for example, Harlow and Lane, *supra*, 1988) or obtained commercially.

The term "detectable secondary antibody" means a secondary antibody, as defined above, that can be detected or measured by analytical methods. Thus, the term secondary antibody includes an antibody labeled directly or indirectly with a detectable marker that can be detected or measured and used in a convenient assay such as an enzyme-linked immunosorbent assay, radioimmunoassay, radial immunodiffusion assay or Western blotting assay. A secondary antibody can be labeled, for example, with an enzyme, radioisotope, fluorochrome or chemiluminescent marker. In addition, a secondary antibody can be rendered detectable using a biotin-avidin linkage such that a detectable marker is associated with the secondary antibody. Labeling of the secondary antibody, however, should not impair binding of the secondary antibody to the I-2 polypeptide. If desired, a multiple antibody system can be used as the secondary antibody as discussed above. In such a system, at least

one of the antibodies is capable of binding the primary anti-I-2 antibody and at least one of the antibodies can be readily detected or measured by analytical methods.

A secondary antibody can be rendered detectable by labeling with an enzyme such as horseradish peroxidase (HRP), alkaline phosphatase (AP), β-galactosidase or urease, for example. A horseradish-peroxidase detection system can be used, for example, with the chromogenic substrate tetramethylbenzidine (TMB), which yields a soluble product in the presence of hydrogen peroxide that is detectable by measuring absorbance at 450 nm. An alkaline phosphatase detection system can be used with the chromogenic substrate *p*-nitrophenyl phosphate, for example, which yields a soluble product readily detectable by measuring absorbance at 405 nm. Similarly, a β-galactosidase detection system can be used with the chromogenic substrate *o*-nitrophenyl-β-D-galactopyranoside (ONPG), which yields a soluble product detectable by measuring absorbance at 410 nm, or a urease detection system can be used with a substrate such as urea-bromocresol purple (Sigma Immunochemicals, St. Louis, MO). A secondary antibody can be linked to an enzyme by methods well known in the art (Harlow and Lane, *supra*, 1988) or can be obtained from a number of commercial sources. For example, goat F(ab')2 anti-human IgG-alkaline phosphatase is a useful detectable secondary antibody that can be purchased from Jackson Immuno-Research (West Grove, PA).

A secondary antibody also can be rendered detectable by labeling with a fluorochrome. Such a fluorochrome emits light of ultraviolet or visible wavelength after excitation by light or another energy source. DAPI, fluorescein, Hoechst 33258, R-phycocyanin,

B-phycoerythrin, R-phycoerythrin, rhodamine, Texas red or lissamine, for example, is a fluorochrome that can be linked to a secondary antibody and used to detect the presence or absence of a complex. A particularly useful fluorochrome is fluorescein or rhodamine. Methods of conjugating and using these and other suitable fluorochromes are described, for example, in Van Vunakis and Langone, Methods in Enzymology, Volume 74, Part C (1991), which is incorporated herein by reference. A secondary antibody linked to a fluorochrome also can be obtained from commercial sources. For example, goat $F(ab')_2$ anti-human IgG-FITC is available from Tago Immunologicals (Burlingame, CA).

A secondary antibody also can be labeled with a chemiluminescent marker. Such a chemiluminescent secondary antibody is convenient for sensitive, non-radioactive detection of a complex containing an I-2 polypeptide and can be obtained commercially from various sources such as Amersham Lifesciences, Inc. (Arlington Heights, IL).

A secondary antibody further can be rendered detectable by labeling with a radioisotope. An iodine-125 labeled secondary antibody is a particularly useful detectable secondary antibody (see, for example, Harlow and Lane, *supra*, 1988).

A signal from a detectable secondary antibody can be analyzed, for example, using a spectrophotometer to detect color from a chromogenic substrate; a fluorometer to detect fluorescence in the presence of light of a certain wavelength; or a radiation counter to detect radiation, such as a gamma counter for detection of iodine-125. For detection of an enzyme-linked

secondary antibody, for example, a quantitative analysis can be made using a spectrophotometer such as an EMAX Microplate Reader (Molecular Devices, Menlo Park, CA) in accordance with the manufacturer's instructions. If desired, the assays of the invention can be automated or performed robotically, and the signal from multiple samples can be detected simultaneously.

The assays of the present invention can be forward, reverse or simultaneous as described in U.S. Patent No. 4,376,110, issued March 8, 1983, to David et al., which is incorporated herein by reference. In the forward assay, each reagent is sequentially contacted with an I-2 polypeptide of the invention. If desired, separation of bound from unbound reagent can be performed before the addition of the next reagent. In a reverse assay, all reagents are pre-mixed prior to contacting with I-2 polypeptide. A modified reverse assay is described in U.S. Patent No. 4,778,751 issued October 18, 1988, to El Shami et al., which is incorporated herein by reference. In a simultaneous assay, all reagents are separately but contemporaneously contacted with an I-2 polypeptide of the invention. A reagent is any component useful in performing the assays of the present invention, for example, the sample, I-2 polypeptide, detectable secondary antibody, washing buffer or other solutions.

Separation steps for the various assay formats described herein, including the removal of unbound secondary antibody from the complex, can be performed by methods known in the art (Harlow and Lane, *supra*, 1988). For example, washing with a suitable buffer can be followed by filtration, aspiration or magnetic separation. If the I-2 polypeptide or an immunoreactive

fragment thereof is immobilized on a particulate support, such as on microparticles, the particulate material can be centrifuged, if desired, followed by removal of wash liquid. If the I-2 polypeptide or an immunoreactive fragment thereof is immobilized on a membrane, filter or well, a vacuum or liquid absorbing apparatus can be applied to the opposite side of the membrane, filter or well to draw the wash liquid away from the complex.

The invention also provides methods of determining susceptibility to IBD in an individual by obtaining a sample from the individual; contacting the sample with an I-2 polypeptide, or immunoreactive fragment thereof, under conditions suitable to form a complex of the I-2 polypeptide, or the immunoreactive fragment thereof, and antibody to the I-2 polypeptide; and detecting the presence or absence of the complex, where the presence of the complex indicates that the individual is susceptible to IBD.

The term "individual," as used herein, means any animal capable of having inflammatory bowel disease, including a human, non-human primate, rabbit, rat or mouse, provided that the animal does not have inflammatory bowel disease as defined by the clinical, endoscopic and histopathologic parameters disclosed herein. A sample to be assayed according to the methods of the invention can be obtained from any such individual.

As used herein, the term "increased susceptibility to IBD" as indicated by the presence of a complex of I-2 polypeptide and antibody to I-2 polypeptide, means a reduced ability to resist IBD-causing factors, as compared with an individual from

whom a sample is obtained that does not form a complex when contacted with I-2 polypeptide or immunoreactive fragment thereof. Increased susceptibility to IBD in an individual does not mean the individual will necessarily develop IBD. However, increased susceptibility to IBD in an individual is associated with an increased probability of having IBD in the future.

The term "immunoreactive fragment," as used in reference to an I-2 polypeptide, means a peptide or polypeptide portion of an I-2 polypeptide that has immunoreactivity as defined by the ability of an anti-I-2 antibody-positive sample to form a complex with the I-2 polypeptide. Thus, the term "immunoreactive fragment of an I-2 polypeptide" as used herein, means a peptide or polypeptide that has an amino acid sequence that is substantially the same as a portion of the amino acid sequence provided as SEQ ID NO: 2 and immunoreactivity as defined by the ability to form a complex with an anti-I-2 antibody-positive sample such as an I-2 reactive serum sample from a Crohn's disease patient. In general, an immunoreactive fragment has from about three amino acids to the full-length of an I-2 polypeptide. An immunoreactive fragment of an I-2 polypeptide can have, for example, at least 5, 8, 10, 12, 15, 18, 20 or 25 amino acids. For example, an immunoreactive fragment of an I-2 polypeptide can be from five to fifty amino acids, from eight to fifty amino acids, or from ten to fifty amino acids. More preferably, an immunoreactive fragment has from eight to twenty amino acids or from ten to twenty amino acids. Most preferably, an immunoreactive fragment has from twelve to twenty amino acids or from fifteen to twenty amino acids.

An immunoreactive fragment of an I-2 polypeptide can be identified by the ability to form a complex with an I-2 reactive sample, for example, an I-2 reactive CD patient serum sample. For example, an immunoreactive fragment of an I-2 polypeptide can be identified by its ability to form a complex when contacted with I-2 reactive CD sera. Assays for the formation of a complex between an antigen and anti-I-2 serum sample are disclosed herein. An ELISA assay can be particularly useful in identifying an immunoreactive fragment of an I-2 polypeptide (see Example IIB).

Identification of a microbial sequence associated with IBD implicates microbes in the pathogenesis of IBD and provides a valuable reagent for ameliorating inflammatory bowel disease. Furthermore, as disclosed herein, the I-2 polypeptide can elicit a T cell response, as demonstrated by the proliferation of murine T cells in response to a GST-I-2 fusion polypeptide (SEQ ID NO: 2), indicating that the I-2 polypeptide antigen can contribute to the etiology of inflammatory bowel disease. Based on identification and isolation of the IBD-associated I-2 sequence, there are provided methods of inducing tolerance in a patient with IBD as well as methods of preventing IBD in a healthy individual.

Thus, the present invention provides methods of inducing tolerance in a patient with inflammatory bowel disease by administering an effective dose of I-2 polypeptide, or tolerogenic fragment thereof, to the patient with IBD. The methods of the invention can be particularly useful for treating a patient having Crohn's disease. In a method of the invention for inducing tolerance, the I-2 polypeptide to be administered can

have, for example, the amino acid sequence of SEQ ID NO: 2.

As used herein, the term "patient with inflammatory bowel disease" means a patient having Crohn's disease or ulcerative colitis.

As used herein, the term "effective dose" means the amount of an I-2 polypeptide, or a tolerogenic fragment thereof, useful for inducing tolerance in a patient with IBD. For induction of oral tolerance, for example, multiple smaller oral doses can be administered or a large dose can be administered. Such doses can be extrapolated, for example, from the induction of tolerance in animal models (see, for example, Trentham et al., Science 261:1727-1730 (1993), which is incorporated herein by reference).

An effective dose of an I-2 polypeptide or a tolerogenic fragment thereof for inducing tolerance can be administered by methods well known in the art. Oral tolerance is well-recognized in the art as a method of treating autoimmune disease (see, for example, Weiner, Hospital Practice, pp. 53-58 (Sept. 15, 1995), which is incorporated herein by reference). For example, orally administered autoantigens suppress several experimental autoimmune models in a disease- and antigen-specific fashion; the diseases include experimental autoimmune encephalomyelitis, uveitis, and myasthenia, collagen- and adjuvant-induced arthritis, and diabetes in the NOD mouse (see, for example, Weiner et al., Ann. Rev. Immunol. 12:809-837 (1994), which is incorporated herein by reference). Furthermore, clinical trials of oral tolerance have produced positive results in treating multiple sclerosis, rheumatoid arthritis and uveitis. In

addition, parenteral administration of an I-2 polypeptide, or a tolerogenic fragment thereof, can be used to induce tolerance. Subcutaneous injection, for example, can be used to deliver an I-2 polypeptide, or a tolerogenic fragment thereof, to an IBD patient, for example, a patient having Crohn's disease (Johnson, Ann. Neurology 36(suppl.):S115-S117 (1994), which is incorporated herein by reference).

The term "tolerogenic fragment," as used in reference to an I-2 polypeptide of the invention, means a peptide or polypeptide portion of the polypeptide that has tolerogenic activity as defined by its ability either alone, or in combination with another molecule, to produce a decreased immunological response. Thus, a tolerogenic fragment of an I-2 polypeptide is a peptide or polypeptide that has substantially the same amino acid sequence as a portion of SEQ ID NO: 2 and tolerogenic activity as defined by its ability either alone, or in combination with another molecule, to produce a decreased immunological response. A tolerogenic fragment of an I-2 polypeptide can have from about three amino acids to about 90 amino acids. A tolerogenic fragment of an I-2 polypeptide can have, for example, at least 5, 8, 10, 12, 15, 18, 20 or 25 amino acids. For example, a tolerogenic fragment of an I-2 polypeptide can have from five to fifty amino acids, from eight to fifty amino acids, or from ten to fifty amino acids. More preferably, a tolerogenic fragment has from eight to twenty amino acids or from ten to twenty amino acids. Most preferably, a tolerogenic fragment has from twelve to twenty amino acids or from fifteen to twenty amino acids.

A tolerogenic fragment of an I-2 polypeptide can be identified using a variety of assays, including *in*

vitro assays such as T-cell proliferation or cytokine secretion assays and *in vivo* assays such as the induction of tolerance in murine models of inflammatory bowel disease. T-cell proliferation assays, for example, are well recognized in the art as predictive of tolerogenic activity (see, for example, Miyahara et al., Immunol. 86:110-115 (1995) or Lundin et al, J. Exp. Med. 178:187-196 (1993), each of which is incorporated herein by reference). A T-cell proliferation assay can be performed by culturing T-cells with irradiated antigen-presenting cells, such as normal spleen cells, in microtiter wells for 3 days with varying concentrations of the fragment of an I-2 polypeptide to be assayed; adding ^{3}H-thymidine; and measuring incorporation of ^{3}H-thymidine into DNA. In such an assay, a fragment of an I-2 polypeptide can be tested for activity, for example, at concentrations of 20 µg/ml and 40 µg/ml.

A tolerogenic fragment of an I-2 polypeptide can be identified using a T-cell cytokine secretion assay known in the art. For example, T cells can be cultured with irradiated antigen-presenting cells in microtiter wells with varying concentrations of the fragment of interest and, after three days, the culture supernatants can be assayed for IL-2, IL-4 or IFN-γ as described in Czerinsky et al., Immunol. Rev. 119:5-22 (1991), which is incorporated herein by reference.

Primary T-cells for use in a T-cell proliferation assay or cytokine secretion assay, for example, can be isolated from lamina propria or peripheral blood. In addition, a convenient source of T-cells for use in an *in vitro* assay for tolerogenic activity can be a T-cell line established from an IBD patient such as a Crohn's disease patient, from a murine

model of IBD or from a healthy animal immunized with an I-2 polypeptide of the invention. A preferred source of T-cells for use in identifying a tolerogenic fragment of an I-2 polypeptide is a Crohn's disease patient.

A T-cell line can be established from a patient with CD or UC, for example, by culturing T lymphocytes with about 1 µg/ml recombinant I-2 polypeptide or GST-I-2, in the presence of low concentrations of growth-supporting IL-2 (about 10 µg/ml). A T-cell line can be established by culturing T lymphocytes with antigen-presenting cells and feeding the cells on an alternating four to five day cycle with either IL-2 and I-2 polypeptide or IL-2 alone as described in Nanda et al., J. Exp. Med. 176:297-302 (1992), which is incorporated herein by reference. A cell line that develops into a reliably proliferating cell line dependent on the presence of I-2 polypeptide is particularly useful in identifying tolerogenic fragments of I-2. The establishment of T-cell lines from small intestinal mucosa is described, for example, in Lundin et al., *supra*, 1993. T cell lines dependent upon the presence of an I-2 polypeptide and useful for identifying I-2 tolerogenic fragments can be prepared similarly.

A tolerogenic fragment of an I-2 polypeptide also can be identified by its ability to induce tolerance *in vivo*, as indicated by a decreased immunological response, which can be a decreased T-cell response, such as a decreased proliferative response or cytokine secretion response as described above, or a decreased antibody titer to the antigen. A neonatal or adult mouse can be tolerized with a fragment of an I-2 polypeptide, for example, and a T-cell response or anti-I-2 polypeptide antibody titer can be assayed after

challenging by immunization. For example, a neonatal mouse can be tolerized within 48 hours of birth by intraperitoneal administration of about 100 µg of a fragment of an I-2 polypeptide emulsified with incomplete Freund's adjuvant and subsequently immunized with I-2 polypeptide at about 8 weeks of age (see, for example, Miyahara, *supra*, 1995). An adult mouse can be tolerized intravenously with about 0.33 mg of a fragment of an I-2 polypeptide, administered daily for three days (total dose 1 mg), and immunized one week later with an I-2 polypeptide. A decreased T-cell response, such as decreased proliferation or cytokine secretion, which indicates tolerogenic activity, can be measured using T-cells harvested 10 days after immunization. In addition, a decreased anti-I-2 polypeptide antibody titer, which also indicates tolerogenic activity, can be assayed using blood harvested 4-8 weeks after immunization. Methods for assaying a T-cell response or anti-I-2 polypeptide antibody titer are described above and are well known in the art.

A tolerogenic fragment of an I-2 polypeptide also can be identified using a murine model of inflammatory bowel disease. Neonatal or adult mice having IBD-like disease can be tolerized with a fragment of an I-2 polypeptide as described above, and the T-cell response or anti-I-2 polypeptide antibody titer assayed. A decreased T-cell response or decreased antibody titer to the antigen indicates a decreased immunological response and, thus, serves to identify a tolerogenic fragment of an I-2 polypeptide. In addition, a tolerogenic fragment of an I-2 polypeptide can be identified by the ability to reduce the frequency, time of onset or severity of colitis in a murine model of IBD.

Several well-accepted murine models of inflammatory bowel disease can be useful in identifying a tolerogenic fragment of an I-2 polypeptide of the invention. For example, mice with target disruption of the genes encoding the alpha subunit of the G-protein Gi2, are a well known model exhibiting features of human bowel disease (Hornquist et al., J. Immunol. 158:1068-1077 (1997); Rudolph et al., Nat. Genet. 10:143-150 (1995), each of which is incorporated herein by reference). Mice deficient in IL-10 as described in Kühn et al., Cell 75:263-274 (1993), which is incorporated herein by reference, and mice deficient in IL-2 as described in Sadlack et al., Cell 75:253-261 (1993), which is incorporated herein by reference, also have colitis like disease and are useful in identifying a tolerogenic fragment of an I-2 polypeptide of the invention. Furthermore, mice with mutations in T cell receptor (TCR) α, TCR β, TCR β x δ, or the class II major histocompatiblility complex (MHC) as described in Mombaerts et al., Cell 75:275-282 (1993), which is incorporated herein by reference, develop inflammatory bowel disease and, thus, are useful in identifying a tolerogenic fragment of an I-2 polypeptide. Similarly, a fragment can be assayed for tolerogenic activity in a SCID mouse reconstituted with CD45RB $CD4^+$ T-cells, which is a well-accepted model of inflammatory bowel disease, as described in Powrie et al., Immunity 1:553-562 (1994), which is incorporated herein by reference. Additional animal models of IBD also are well known in the art (see, for example, Podolsky, Acta Gastroenterol. Belg. 60:163-165 (1997); and Bregenholt et al., APMIS 105: 655-662 (1997), each of which is incorporated herein by reference). Thus, a tolerogenic fragment of an I-2 polypeptide can be readily identified by an *in vitro* or

in vivo assay disclosed herein or by another assay well known in the art.

An immunoreactive or tolerogenic fragment of an I-2 polypeptide can be identified by screening a large collection, or library, of peptides of interest or random peptides for immunoreactivity or tolerogenic activity. For example, a panel of peptides spanning the entire sequence of an I-2 polypeptide can be screened for immunoreactivity or tolerogenic activity as described above. Such a panel can be a panel of 15-mer peptides spanning the sequence of the I-2 polypeptide (SEQ ID NO: 2), each overlapping by three or five residue shifts using the Mimotope cleavable pin technology (Cambridge Research Biochemicals, Wilmington, DE), as described by Geysen et al., Science 235:1184 (1987), which is incorporated herein by reference. The panel is subsequently screened for immunoreactivity or tolerogenic activity using one of the assays described above (see, for example, Miyahara et al., *supra*, 1995, which is incorporated herein by reference). A library of peptides to be screened also can be a population of peptides related in amino acid sequence to SEQ ID NO: 2 but having one or more amino acids that differ from SEQ ID NO: 2.

Additional peptides to be screened include, for example, tagged chemical libraries of peptides and peptidomimetic molecules. Peptide libraries also comprise those generated by phage display technology. Phage display technology includes the expression of peptide molecules on the surface of phage as well as other methodologies by which a protein ligand is or can be associated with the nucleic acid which encodes it. Methods for production of phage display libraries, including vectors and methods of diversifying the

population of peptides which are expressed, are well known in the art (see, for example, Smith and Scott, Methods Enzymol. 217:228-257 (1993); Scott and Smith, Science 249:386-390 (1990); and Huse, WO 91/07141 and WO 91/07149, each of which is incorporated herein by reference). These or other well known methods can be used to produce a phage display library which can be screened, for example, with one of the disclosed assays for immunoreactivity or tolerogenic activity. If desired, a population of peptides can be assayed for activity *en masse*. For example, to identify an immunoreactive fragment of an I-2 polypeptide, a population of peptides can be assayed for the ability to form a complex with a sample containing anti-I-2 polypeptide reactivity; the active population can be subdivided and the assay repeated in order to isolate the immunoreactive fragment from the population.

An immunoreactive or tolerogenic fragment of an I-2 polypeptide also can be identified by screening, for example, fragments of the polypeptide produced by chemical or proteolytic cleavage. Methods for chemical and proteolytic cleavage and for purification of the resultant protein fragments are well known in the art (see, for example, Deutscher, Methods in Enzymology, Vol. 182, "Guide to Protein Purification," San Diego: Academic Press, Inc. (1990), which is incorporated herein by reference). For example, a chemical such as cyanogen bromide or a protease such as trypsin, chymotrypsin, V8 protease, endoproteinase Lys-C, endoproteinase Arg-C or endoproteinase Asp-N can be used to produce convenient fragments of an I-2 polypeptide that can be screened for immunoreactivity or tolerogenic activity using one of the assays disclosed herein.

As used herein, the term "fragment" means a peptide, polypeptide or compound containing naturally occurring amino acids, non-naturally occurring amino acids or chemically modified amino acids. An immunoreactive or tolerogenic fragment of an I-2 polypeptide also can be a peptide mimetic, which is a non-amino acid chemical structure that mimics the structure of a peptide having an amino acid sequence, provided that the peptidomimetic retains immunoreactivity or tolerogenic activity, as defined herein. Such a mimetic generally is characterized as exhibiting similar physical characteristics such as size, charge or hydrophobicity in the same spatial arrangement found in its peptide counterpart. A specific example of a peptide mimetic is a compound in which the amide bond between one or more of the amino acids is replaced, for example, by a carbon-carbon bond or other bond well known in the art (see, for example, Sawyer, Peptide Based Drug Design, ACS, Washington (1995), which is incorporated herein by reference).

As used herein, the term "amino acid" refers to one of the twenty naturally occurring amino acids, including, unless stated otherwise, L-amino acids and D-amino acids. The term amino acid also refers to compounds such as chemically modified amino acids including amino acid analogs, naturally occurring amino acids that are not usually incorporated into proteins such as norleucine, and chemically synthesized compounds having properties known in the art to be characteristic of an amino acid, provided that the compound can be substituted within a peptide such that it retains immunoreactivity or tolerogenic activity. Examples of amino acids and amino acids analogs are listed in Gross and Meienhofer, The Peptides: Analysis, Synthesis,

Biology, Academic Press, Inc., New York (1983), which is incorporated herein by reference. An amino acid also can be an amino acid mimetic, which is a structure that exhibits substantially the same spatial arrangement of functional groups as an amino acid but does not necessarily have both the α-amino and α-carboxyl groups characteristic of an amino acid.

An immunoreactive or tolerogenic fragment of an I-2 polypeptide can be produced or synthesized using methods well known in the art. Such methods include recombinant DNA methods and chemical synthesis methods for production of a peptide. Recombinant methods of producing a peptide through expression of a nucleic acid sequence encoding the peptide in a suitable host cell are well known in the art and are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed, Vols 1 to 3, Cold Spring Harbor Laboratory Press, New York (1989), which is incorporated herein by reference. The sequence of a nucleic acid molecule encoding an I-2 polypeptide is disclosed herein as SEQ ID NO: 1.

An immunoreactive or tolerogenic fragment of an I-2 polypeptide also can be produced by chemical synthesis, for example, by the solid phase peptide synthesis method of Merrifield et al., J. Am. Chem. Soc. 85:2149 (1964), which is incorporated herein by reference. Standard solution methods well known in the art also can be used to synthesize an immunoreactive or tolerogenic fragment useful in the invention (see, for example, Bodanszky, Principles of Peptide Synthesis, Springer-Verlag, Berlin (1984) and Bodanszky, Peptide Chemistry, Springer-Verlag, Berlin (1993), each of which is incorporated herein by reference). A newly

synthesized peptide can be purified, for example, by high performance liquid chromatography (HPLC), and can be characterized using, for example, mass spectrometry or amino acid sequence analysis.

The present invention also provides tolerogenic compositions that contain an I-2 polypeptide and are useful in inducing tolerance in a patient with IBD. In particular, the invention provides compositions including an I-2 polypeptide having substantially the same amino acid sequence as SEQ ID NO: 2, or tolerogenic fragment thereof, combined with a tolerogizing molecule. In a composition of the invention, the I-2 polypeptide can have, for example, the amino acid sequence SEQ ID NO: 2. A tolerogenic fragment useful in a composition of the invention can have, for example, at least ten contiguous amino acids of SEQ ID NO: 2.

Various molecules are known in the art to cause, promote or enhance tolerance. See, for example, U.S. Patent No. 5,268,454, and citations therein, which are incorporated herein by reference. As used herein, the term "tolerogizing molecule" means a molecule, compound or polymer that causes, promotes or enhances tolerogenic activity when combined with an I-2 polypeptide of the invention, or fragment thereof. A tolerogizing molecule can be, for example, conjugated to an I-2 polypeptide. Such tolerogizing molecules include, for example, polyethylene glycol and are well known in the art (see, for example, U.S. Patent No. 5,268,454, *supra*).

The invention also provides methods of preventing IBD in an individual by administering an effective dose of an I-2 polypeptide, or tolerogenic

fragment thereof, to the individual. The methods of the invention are particularly useful for preventing IBD, for example, Crohn's disease in an individual having increased susceptibility to IBD. Such methods can be particularly useful for preventing IBD when an effective dose of the antigen or tolerogenic fragment is administered to a newborn individual.

Enteric bacteria have been shown to play a role in the pathogenesis of several diseases. For example, *H. pylori* has been implicated in pathogenesis of peptic ulcer disease, and antibiotics against *H. pylori* can be used to effectively treat this disease (see, for example, Sontag, Am. J. Gastroenterol. 92:1255-1261 (1997); and Pipkin et al., Helicobactor. 2:159-171 (1997), each of which is incorporated herein by reference). In Crohn's disease, intestinal inflammation is notable for its frequent responsiveness to antibiotics and susceptibility to bacterial fecal flow (Gui et al., J. Antimicrob. Chemother. 39:393-400 (1997); Prantera et al., Am. J. Gastroenterol. 91:328-332 (1996); and Janowitz et al., Inflamm. Bowel. Dis. 4:29-39 (1998), each of which is incorporated herein by reference). Common intestinal colonists and novel pathogens have been implicated in CD by direct detection or by disease-associated anti-microbial immune responses (Blaser et al., Gastroenterology 87:888-894 (1984); Elsaghier et al., Clin. Exp. Immunol. 90:503-508 (1992); Del Prete et al., J. Microbiol. Methods 33:105-114 (1998); Metcalf, British Medical Journal 316:166 (1998), each of which is incorporated herein by reference; and Sendid et al., *supra*, 1996). Furthermore, in most animal models of chronic colitis, lumenal micro-organisms are a necessary co-factor for disease. Dietary antigens also have been implicated in IBD pathogenesis (Sonnenberg, Gut

31:1037-1040 (1990); Davidson et al., Clin. Exp. Immunol. 35:147-148 (1979); and Knoflach et al., Gastroenterology 92:479-485 (1987), each of which is incorporated herein by reference). Despite extensive research, the pathogenic organism remains to be identified.

As disclosed herein, colonic microbes harbored in inflamed lesions in Crohn's disease patients contain a nucleic acid sequence encoding the I-2 polypeptide antigen (SEQ ID NO: 2). Based on this finding, the microbial organism can be isolated and used for discovery of agents that reduce the viability or growth of the organism, thereby diminishing the immune stimulus contributing to Crohn's and ameliorating symptoms of the disease.

Thus, the invention provides a method of identifying an agent useful in treating inflammatory bowel disease. The method includes the steps of obtaining a specimen of an enteric microbe from a patient with inflammatory bowel disease; isolating from the specimen a microbial species that includes a nucleic acid molecule encoding an I-2 polypeptide; contacting the microbial species with an agent; and assaying for reduced growth or viability of the microbial species as compared to the growth or viability in the absence of the agent, where the reduced growth or viability of the microbial species indicates that the agent is an agent useful in treating inflammatory bowel disease. The methods of the invention can be particularly useful for screening agents which are antibiotics and for identifying agents for the treatment of Crohn's disease. One skilled in the art understands that the microbial species which is contacted with an agent in the methods of the invention can be a single microbial species or can be a mixture of two or

more microbial species, where at least one species contains a nucleic acid sequence encoding an I-2 polypeptide.

Isolation of a microbial species that includes a nucleic acid molecule encoding an I-2 polypeptide can be performed by culturing the I-2 positive specimen on a variety of mediums and under aerobic and anaerobic conditions as described in Example IV; isolates are subsequently screened for the presence of the I-2 sequence using, for example, PCR analysis. Exemplary culture conditions are set forth in Figure 7.

As used herein, the term "agent" means a biological or chemical compound such as a simple or complex organic molecule, a peptide, a protein, an antibody, a lipid or an oligonucleotide.

An agent identified by the methods of the invention reduces the viability or growth of a microbial species that contains a nucleic acid molecule encoding an I-2 polypeptide. Thus, an agent useful in treating IBD can be an agent that functions to kill or slow the growth of a microbial species that contains a nucleic acid molecule encoding an I-2 polypeptide. An agent useful in treating IBD can be a bacteriostatic or bacteriocidal agent such as a bacterial antibiotic, which is a molecule that is produced by a microorganism or a plant, or a chemical derivative of such a molecule, that can reduce the growth or viability of a bacterial species that contains a nucleic acid molecule encoding an I-2 polypeptide. One skilled in the art understands that an agent useful in treating IBD can function by a variety of mechanisms, for example, by inhibiting microbial protein synthesis, inhibiting microbial DNA synthesis, inhibiting

microbial cell wall synthesis or inhibiting synthesis of an essential nutrient of a microbial species that contains a nucleic acid molecule encoding an I-2 polypeptide. Such an agent can selectively reduce the viability or growth of a particular microbial species that contains a nucleic acid molecule encoding an I-2 polypeptide. An agent useful in treating IBD also can have activity in reducing the growth or viability of a broad spectrum of microbes. One skilled in the art understands that, preferably, an agent useful in treating IBD reduces the growth or viability of a microbial species that contains a nucleic acid molecule encoding an I-2 polypeptide without significantly altering the growth or viability of mammalian cells, especially human cells.

As used herein, the term "agent useful in treating IBD" means an agent that reduces the severity, frequency, or time of onset of one or more symptoms of inflammatory bowel disease.

Although animal models of inflammatory bowel disease are known, these models do not involve an antigen associated with human IBD. Based on the disclosed isolation of the IBD-associated I-2 polypeptide antigen (SEQ ID NO: 2) and the demonstration that murine T cells are responsive to this antigen (see Example III), the invention provides novel animal models for IBD, in which disease is initiated with the I-2 antigen associated with human disease. Thus, superior IBD animal models are provided for identifying new IBD therapeutics, including antibiotics, anti-inflammatories and other drugs.

In particular, the invention provides a method of identifying an agent useful in treating inflammatory bowel disease by administering an I-2 polypeptide to a

non-human animal, whereby one or symptoms of IBD are exhibited; administering an agent to the non-human animal; and assaying the level of the one or more symptoms characteristic of IBD, where a reduction in the level of the one or more symptoms as compared to a control level indicates that the agent is an agent useful in treating IBD. The methods of the invention can be applied, for example, to identification of agents useful in treating Crohn's disease. The I-2 polypeptide administered can have, for example, the amino acid sequence SEQ ID NO: 2. A non-human animal particularly useful in the methods of the invention can be, for example, a mouse deficient in Gαi2, TCRα or IL-10.

As set forth above, the term "agent" means a biological or chemical compound such as a simple or complex organic molecule, a peptide, a protein, an antibody, a lipid or an oligonucleotide. An agent can be, for example, an antibiotic or anti-inflammatory compound.

The methods of the invention rely on the use of a non-human animal, which can exhibit one or more symptoms of inflammatory bowel disease in response to an antigenic trigger. Any non-human animal model of disease can be useful in the methods of the invention including non-human primate, rat or mouse model of IBD. A variety of mouse models of IBD well known in the art can be particularly useful in the invention (see, for example, Podolsky, *supra*, 1997; Bregenholt et al., *supra*, 1997). Such mouse models include those described hereinabove, for example, mice deficient in Gαi2 (Gαi2 -/-; Rudolph et al., *supra*, 1995); mice deficient in IL-10 (IL-10 -/-; Kühn et al., *supra*, 1993); mice deficient in TCRα (TCRα -/-); mice deficient in TCRβ (TCRβ -/-); mice deficient

in keratin 8; mice deficient in IL-2 (IL-2 -/-; Sadlack, *supra,* 1993); and SCID mice reconstituted with CD45RB $CD4^+$ T cells (Powrie et al., *supra,* 1994). Non-human animal models useful in the methods of the invention also include animals expressing transgenes for human HLA-B27 (with β2-microglobulin) or animals expressing a dominant negative construct that functionally blocks N-cadherin (Podolsky, *supra,* 1997).

One skilled in the art understands that, in order to initiate one or more symptoms of IBD in a non-human animal model, an I-2 polypeptide of the invention can be administered by a variety of routes. Preferred administration of an I-2 polypeptide to initiate one or more symptoms of IBD in a non-human animal model of the disease is by enema administration or by administering a cellular composition in which the I-2 polypeptide is expressed using *E. coli.*

Local administration of free antigen can be achieved by enema, for example, as described in Mahler et al., Am. J. Physiol. 274:G544-G551 (1998). An I-2 polypeptide also can be administered by colonization of the mouse colon with antigen-bearing microorganisms, for example, *E. coli* (Cahill et al., Infect Immuno., 65:3126-3131 (1997)) or another bacterial species (Kullberg et al., Infect. Immun. 66:5157-5166 (1998); Li et al., Infect. Immun. 66:5477-5484 (1998); and Cahill, *supra,* 1997). In a non-human animal model of IBD, one or more symptoms of IBD also can be induced using a T cell line reactive with a bacterial antigen, where the T cell line is derived from a colitis susceptible mouse strain. Such a T cell line can be, for example, an *E. coli* reactive T cell line produced from C3H/HeJBir mice (Cong et al., J. Exp. Med. 187:855-864 (1998)). A C.B-17Scid mouse

also can be colonized with antigen-bearing bacteria, either naturally or by intra-intestinal infection (Kullberg et al., *supra*, 1998; Li et al., *supra*, 1998; and Cong et al., *supra*, 1998). Transfer of a T cell line into such a mouse results in a robust antigen-specific inflammatory bowel disease model (Kullberg et al., *supra*, 1998; and Cong et al., *supra*, 1998).

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

IDENTIFICATION OF THE I-2 and I-1 TARGET ANTIGENS

This example describes isolation of novel microbial nucleic acid sequences encoding the I-1 and I-2 polypeptides.

A. Representational difference analysis

Representational difference analysis (RDA), which uses PCR driven subtractive cloning to identify DNA sequences found preferentially in an infected area, was utilized to examine sequences differentially present in involved versus uninvolved CD mucosa.

RDA was performed essentially as described in Chang et al., Science 266:1865-1869 (1994), which is incorporated herein by reference. Briefly, mononuclear cells from the lamina propria of a Crohn's disease patient were isolated from an area with ulcerations (tester cells) and from an area macroscopically free of disease (driver cells), and DNA purified from each cell population. Using RDA, the DNA sequences from the area free of disease (driver DNA) were subtracted from the DNA

found at the site of ulceration (tester DNA), leaving sequences preferentially found in the inflamed area.

Four sequences were obtained, two of which were human sequences. The remaining two sequences, designated I-1 and I-2, showed no significant homology to any human genes. The I-2 nucleic acid sequence (SEQ ID NO: 1) and encoded polypeptide (SEQ ID NO: 2) are shown in Figure 1A. The I-1 nucleic acid sequence (SEQ ID NO: 3) and two encoded open reading frames (SEQ ID NOS: 4 and 5) are shown in Figure 1B.

B. PCR analysis of archived paraffin embedded tissue samples

Paraffin embedded tissue samples from CD patients, UC patients, or non-IBD controls were subject to PCR analysis. Briefly, sample was amplified with the I-2 specific primers 5'-CCGTGGGCATCCAGTCCG-3' (SEQ ID NO: 9) and 5'-TCTGCTCATACACGTCACG-3' (SEQ ID NO: 10) using standard PCR conditions with a final concentration of 4 mM $MgCl_2$. In particular, the reactions consisted of 26.75 µl H_2O; 5.0 µl 10X PCR Buffer; 5.0 µl 25 mM $MgCl_2$; 1.0 µl 10 mM each dNTP; 1.0 µl each of SEQ ID NOS: 9 and 10; and 0.25 µl Taq polymerase (5 units/µl). The reactions were incubated at 94°C for 5 minutes; followed by 39 cycles of 94°C for 30 seconds/65°C for 30 seconds and 72°C for 30 seconds, followed by a final 5 minute extension at 72°C. Reactions were analyzed on 2% agarose, with the presence of the I-2 sequence indicated by the expected 285 bp fragment.

As shown in Figure 3, the I-2 sequence (SEQ ID NO: 1) was present significantly more frequently in involved CD samples than in UC samples or non-IBD

samples. These results indicate that the I-2 polypeptide antigen (SEQ ID NO: 2) is associated with inflammatory bowel disease, in particular, with Crohn's disease.

EXAMPLE II

DIFFERENTIAL REACTIVITY OF IBD PATIENT AND NORMAL SERA TO THE I-2 POLYPEPTIDE

This example demonstrates that the I-2 polypeptide is differentially reactive with Crohn's disease patient sera as compared to normal sera.

A. GST-I-2 fusion protein

The full-length I-2 encoding nucleic acid sequence (SEQ ID NO: 1) was cloned into the GST expression vector pGEX. After expression in *E. coli*, the protein was purified on a GST column. The purified protein was shown to be of the expected molecular weight by silver staining, and had anti-GST reactivity upon western analysis.

B. ELISA analysis

ELISA analysis was performed with GST-I-2 (SEQ ID NO: 2) fusion polypeptide using diluted patient or normal serum. Reactivity was determined after subtracting reactivity to GST alone. Varying dilutions of CD sera and sera from normal individuals were assayed for IgG reactivity to the GST-I-2 fusion polypeptide. As shown in Figure 4A, dilutions of 1:100 to 1:1000 resulted in significantly higher anti-I-2 polypeptide reactivity for the CD sera as compared to normal sera. These results indicate that the I-2 polypeptide (SEQ ID NO: 2)

is differentially reactive with Crohn's disease sera as compared to normal sera.

Serum IgA reactivity of UC, CD and normal sera to the I-2 polypeptide (SEQ ID NO: 2) was assayed as described below. As shown in Figure 4B, using a cutoff that is two standard deviations above the mean value for the normal population, nine of ten CD values were positive, while none of the normal serum samples were positive. Furthermore, seven of ten Crohn's disease patients showed an OD_{405} greater than 0.3, while none of the UC or normal samples were positive by this measure. These results indicate that immunoreactivity to the I-2 polypeptide, in particular, IgA immunoreactivity, can be used to diagnose Crohn's disease.

Human IgA and IgG antibodies that bind the I-2 polypeptide (SEQ ID NO: 2) were detected by direct ELISA assays essentially as follows. Plates (Immulon 3; DYNEX Technologies; Chantilly, VA) were coated overnight at 4°C with 100 μl/well GST-I-2 fusion polypeptide (5 μg/ml in borate buffered saline, pH 8.5). After three washes in 0.05% Tween 20 in phosphate buffered saline (PBS), the plates were blocked with 150 μl/well of 0.5% bovine serum albumin in PBS, pH 7.4 (BSA-PBS) for 30 minutes at room temperature. The blocking solution was then replaced with 100 μl/well of Crohn's disease serum, ulcerative colitis serum, or normal control serum, diluted 1:100. The plates were then incubated for 2 hours at room temperature and washed as before. Alkaline phosphatase conjugated secondary antibody [goat anti-human IgA (α-chain specific), Jackson ImmunoResearch, West Grove, PA] was added to the IgA plates at a dilution of 1:1000 in BSA-PBS. For IgG reactivity, alkaline phosphatase conjugated secondary antibody (goat anti-human IgG

(γ-chain specific), Jackson ImmunoResearch) was added. The plates were incubated for 2 hours at room temperature before washing three times with 0.05% Tween 20/PBS followed by another three washes with Tris buffered normal saline, pH 7.5. Substrate solution (1.5 mg/ml disodium P-nitrophenol phosphate (Aresco; Solon, OH) in 2.5 mM $MgCl_2$, 0.01 M Tris, pH 8.6) was added at 100 μl/well, and color allowed to develop for one hour. The plates were then analyzed at 405 nm.

C. Histological analysis

Rabbit anti-I-2 antibodies are prepared using purified GST-I-2 fusion protein as the immunogen. GST binding antibodies are removed by adherence to GST bound to an agarose support (Pierce), and the rabbit sera validated for anti-I-2 immunoreactivity by ELISA analysis.

Slides are prepared from paraffin embedded biopsy specimens from CD, UC and normal controls. Hematoxylin and eosin staining are performed, followed by incubation with I-2 specific antiserum. Binding of antibodies is detected with peroxidase labeled anti-rabbit secondary antibodies (Pierce). The assay is optimized to maximize the signal to background and the distinction between CD and control populations.

EXAMPLE III

REACTIVITY OF $CD4^+$ T CELLS WITH THE I-2 POLYPEPTIDE

This example demonstrates that T cells derived from normal mice proliferate in response to the I-2 antigen (SEQ ID NO: 2).

$CD4^+$ T cells were isolated from normal C57BL/6J mice, and T cell proliferation assayed in response to the I-2 antigen as follows. To prepare the $CD4^+$ T cells, spleens from 8-10 week old female mice were removed and placed into cell suspension. The cells were depleted for B cells and antigen presenting cells (APCs) using nylon wool and then depleted for CD8+ T cells using anti-CD8 magnetic beads. Flow cytometry was used to determine the purity of the $CD4^+$ T cell suspension.

For preparing APCs, spleens from 8-10 week old females are removed, placed into cell suspension, and pulsed overnight with 0, 2, 10, or 15 µg/ml of the I-2 polypeptide. The APCs are irradiated with 3,000 rads before being added to the T cell cultures. To assay for antigen specific proliferation of T cells, 4×10^5 $CD4^+$ T cells/well were incubated with 4×10^5 antigen-pulsed APCs/well in a 96-well flat bottomed tissue culture plate at 37 C in 5% CO_2 humidified air. After varying periods of incubation, 0.5 µCi of [^{3}H]-thymidine was added to each culture for the last 18 hours of incubation. The cells were harvested and proliferation assessed as the amount of [^{3}H]-thymidine into cell DNA by scintillation counting.

As shown in Figure 5, the I-2 polypeptide antigen (SEQ ID NO: 2) stimulates T cell proliferation at concentrations of 5 µg/ml and 25 µg/ml. These results demonstrate that the I-2 polypeptide (SEQ ID NO: 2) associated with human inflammatory bowel disease can elicit a T cell response by murine cells and indicate that an I-2 polypeptide can contribute to the etiology of inflammatory bowel disease.

B. Cytokine expression in response to the I-2 polypeptide

A role for regulatory T cells is supported by studies with $CD45RB^{low}$, $CD28+CD45RB^{low}$ and $CD25+CD45RB^{low}$ cells. Cytokine expression was therefore analyzed in response to the I-2 polypeptide. T cell proliferation cultures were used for cytokine assays. On day 4 of the T cell proliferation assay, supernatants were analyzed for IL-10 and interferon-γ expression. As shown in Figure 6, expression of interferon-γ, a Th1 cytokine regulator, is induced by the GST-I-2 polypeptide (SEQ ID NO: 2). Furthermore, Figure 6 shows that IL-10 expression, which can act to down regulate a Th1 cytokine response, is induced by exposure of $CD4^+$ T cells to as little at 2 µg/ml GST-I-2 polypeptide (SEQ ID NO: 2). These results indicate that an animal model of IBD in which disease is initiated by the I-2 polypeptide antigen (SEQ ID NO: 2) can be a useful model of human disease.

EXAMPLE IV

ISOLATION AND IDENTIFICATION OF THE ORGANISM CONTAINING THE I-2 SEQUENCE

This example describes isolation and identification of the organism containing the I-2 sequence.

Tissue resections from CD patients are obtained from Cedars-Sinai under anaerobic conditions. DNA is extracted from a small piece of tissue using the Qiagen tissue prep kit, and tested for the presence of the I-2 nucleic acid sequence SEQ ID NO: 1 using the PCR assay described above. Cells from positive scoring sections

are cultured under a variety of conditions (see Figure 7), and the isolates catalogued.

After assaying the isolates by PCR for 16S RNA to assure that the target DNA is accessible to amplification, isolates are assayed by PCR for the I-2 nucleic acid sequence (SEQ ID NO: 1) as described in Example I. The 16S RNA of isolates containing the I-2 nucleic acid are sequenced to identify the organism essentially as described in Wilson and Blitchington, Applied and Environ. Microbiol. 62:2273-2278 (1996), which is incorporated herein by reference.

EXAMPLE V

DEMONSTRATION OF I-2 ENTERIC, T CELL SUPERANTIGEN ACTIVITY

This example demonstrates that an I-2-containing microorganism present in normal C57BL/6J mice is a target of a $CD4^+$ T cell response. This example further demonstrates that the I-2 response is dependent on MHC class II-mediated recognition but does not require antigen processing, indicating that I-2 represents a new class of T cell superantigen.

A. Mouse tissues contain the I-2 sequence

To determine if the I-2 sequence is present in normal mouse tissue, genomic DNA was prepared from the distal colon, proximal colon, cecum, distal small intestine, proximal small intestine and stomach of normal C57BL/6J mice. PCR analysis detected the 285 bp fragment characteristic of the I-2 sequence in DNA prepared from the distal small intestine, cecum and proximal colon, a pattern identical to that seen in human samples. In contrast, no detectable I-2 fragment was amplified from

genomic DNA isolated from similar tissues of C57BL/6J mice harboring only defined flora, including a non-pathogenic strain of *Clostridium*. These results indicate that an I-2 expressing microbe is present in mice under conventional conditions, but not in mice containing defined flora.

Mice were maintained as follows. C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Normal C57BL/6J mice greater than eight weeks old were housed in conventional facilities unless otherwise indicated. Both male and female mice were used. Rag-/- mice containing defined flora crossed onto a C57BL/6J background were obtained from the UCLA breeding colony of Dr. W. McBride and housed in specific pathogen free conditions.

I-2 and mouse glyceraldehyde 3-phosphate dehydrogenase (GAPDH) PCR was performed as follows. Mouse genomic DNA was prepared from various tissues by NucleoSpin Tissue kit (Clonetech). The native I-2 and positive control mimic I-2 PCR reactions yielded 285 and 606 bp products, respectively, when amplified with primers SEQ ID NOS: 9 and 10. The negative control PCR reaction (buffer only) did not produce any detectable product. PCR conditions were specific for I-2, as shown by amplification of a 606 bp fragment from a synthetic I-2 template. Similar quantities of DNA were present in each sample as shown by amplification of a 340 bp mouse GAPDH fragment from each genomic DNA sample template. The GADPH primers were 5'-GCCAAGATGTCATTTGAATGG G-3' (SEQ ID NO: 11) and 5'-GAATCCATATAGAGGCTGGG-3' (SEQ ID NO: 12). All reactions were performed at 94^0 C, 30 seconds for 1 cycle; 94^0 C, 30 seconds, 65^0 C, 30 seconds,

72° C, 40 seconds for 39 cycles; and 72° C, 10 minute for 1 cycle.

B. CD4⁺ T cells from normal C57BL/6J and 129Sv mice spontaneously proliferate and produce IL-10 in response to I-2 pulsed antigen-presenting cells

The native response to the I-2 antigen was evaluated in normal mouse strains housed in conventional conditions. Splenic CD4⁺ T cells were isolated from non-immunized C57BL/6J and 129Sv mice, and cultured with syngeneic splenic antigen-presenting cells pretreated with various concentrations of GST-I-2, GST alone, or *Staphylococcus* enterotoxin (SEB). Proliferation was assessed by [^{3}H]-thymidine incorporation, and secretion of various cytokines (IL-10, IFN-γ, TNF-α, and IL-4) was quantitated by ELISA.

CD4⁺ T cells from C57BL/6J mice displayed a dose-dependent proliferative response to GST-I-2. This response was specific for the I-2 sequence, as indicated by a minimal response to GST alone. The positive control for the T cell proliferation assay, *Staphylococcus* enterotoxin (SEB), yielded responses similar in magnitude to the maximal I-2 response. Furthermore, C57BL/6J CD4⁺ T cells secreted the cytokine IL-10 in response to GST-I-2, while several other cytokines were not detectably secreted, including IFN-γ, IL-4, and TNF-α. GST alone did not stimulate secretion of any cytokines, indicating that the response was specific to the I-2 antigen. These results indicate that CD4⁺ T cells from conventionally housed C57BL/6J mice spontaneously proliferate and produce IL-10 in response to antigen-presenting cells that have been previously exposed to I-2.

The proliferation and cytokine secretion assays were performed as follows. Splenic $CD4^+$ T cells were isolated from non-immunized C57BL/6J and 129Sv mice essentially as described in Example III. Antigen-presenting cells were isolated from spleens removed from C57BL/6J or 129Sv mice and placed into cell suspension. The nucleated spleen cells were treated with various concentrations of GST-I-2, GST, or *Staphylococcus* enterotoxin at 2×10^7 cells per 4 ml in 15 ml conical tubes (Corning, Acton, MA) for 12 to 16 hours at 37°C. After washing, the cells were resuspended at 4×10^6 cells/ml in complete media containing RPMI 1640 5% fetal calf serum (FCS), 0.05 mM 2-mercaptoethanol, penicillin, and streptomycin. Antigen-presenting cells were irradiated at 3000 rads (567.7cGy/minute for 5.5 minutes) before addition to T cell cultures.

Syngeneic antigen-presenting cells (4×10^5) were antigen-pulsed with GST alone or GST-I-2 at 0, 1 and 5 µg/ml and SEB (SIGMA) at 1 µg/mL and cultured with 4×10^5 splenic $CD4^+$ T cells from C57BL/6J mice in microtiter wells. GST and GST-I-2 proteins were produced as described in Example II. After 48 hours, cells were assayed for cytokine production by collecting culture supernatants and ELISA assays were performed for IL-4, IL-10, TNF-α, and IFN-γ according to the manufacturer's instructions (R&D; Minneapolis, MN); values are the means of duplicate samples. After 72 hours, cells were assayed for proliferation by pulsing with $[^3H]$-thymidine, and incorporated cpm (mean ± SD) were determined for triplicate cultures essentially as described in Example III.

C. CD4⁺ T cells from mice containing defined flora spontaneously proliferate, but do not produce IL-10 in response to I-2

The response of normal C57BL/6J mice to GST-I-2 was compared to that of C57BL/6J mice containing defined flora and housed under specific pathogen-free conditions. $CD4^+$ T cells isolated from C57BL/6J mice containing defined flora had a proliferative response to the GST-I-2 protein. However, in contrast to the GST-I-2 response observed in the conventionally housed normal C57BL/6J mice, no cytokine response was observed. The cells were proliferation competent as shown with the SEB positive control, and, furthermore, the response was I-2 specific since no proliferative or cytokine response was observed in response to GST alone.

The response of mice containing defined flora to GST-I-2 indicates that the I-2 antigen was able to stimulate $CD4^+$ T cells without prior exposure to antigen. Coupled with the demonstration that the I-2 sequence was not present in C57BL/6J mice containing defined flora, these results indicate that the GST-I-2 response can be a result of superantigen or mitogen stimulation.

D. Both naïve and memory CD4⁺ T cell populations from normal C57BL/6J mice spontaneously proliferate and produce IL-10 in response to I-2

The I-2 response was evaluated using a naïve $CD4^+$ T cell population. $CD4^+$ T cells from conventionally housed C57BL/6J mice were fractionated into $CD62L^{hi}CD44^{low}$ naïve T cells and $CD62L^{low}CD44^{hi}$ memory T cells, and subsequently cultured with GST-I-2, GST alone or SEB pulsed antigen-presenting cells. Both naïve and memory

$CD4^+$ T cells had a strong proliferative and IL-10 response to GST-I-2 (Figures 8A and 8B). The response was specific to I-2 since neither population was responsive to GST alone (Figures 8A and 8B), and both populations were proliferation competent as shown with the SEB positive control. These results demonstrate that GST-I-2 antigen is not a conventional MHC class II processed antigen, but a superantigen or mitogen.

Naïve and memory subpopulations of $CD4^+$ T cells from conventionally housed normal C57BL/6J mice were obtained and analyzed as follows. Naïve $CD4^+$ T cells were obtained from $CD4^+$ T cells, isolated as described in section VB above, and incubated with rat anti-mouse CD44 for 30 minutes on ice. Memory $CD4^+$ T cells were obtained from $CD4^+$ T cells, incubated with rat anti-mouse CD45RB and rat-anti mouse CD62L for 30 minutes on ice. The cells were washed and treated with sheep anti-rat-IgG coated Dynal magnetic beads for 30 minutes on ice. After depletion, the unbound T cells were negatively selected and resuspended at 4×10^6 cells/ml in complete RPMI media containing 5% FCS. Fractionated T cells were assessed for purity by flow cytometry with FITC-conjugated anti-CD4, CD44, CD45RB and CD62L (Pharmingen; San Diego, CA). Populations were typically greater than 85% memory or naive $CD4^+$ T cells.

Fractionated naïve and memory $CD4^+$ T cells from C57BL/6J mice (4×10^5) were cultured in microtiter wells with 4×10^5 syngeneic antigen-presenting cells previously antigen-pulsed with antigen (0, 1, 5 µg/mL; GST; or GST-I-2) or 1 µg/ml positive control SEB as described above. Proliferation and cytokine production were assayed as described above.

E. MHC class II restriction of CD4+ T cell response to I-2

The I-2 response was analyzed for dependence on MHC class II processing. $CD4^+$ T cells were cultured with I-2 pulsed antigen-presenting cells with either monoclonal antibody to $I\text{-}A\text{-}^b$ or $I\text{-}A\text{-}^d$ MHC class II molecules. $Anti\text{-}I\text{-}A\text{-}^b$ antibody blocked the T cell proliferative response to both SEB and GST-I-2 while $anti\text{-}I\text{-}A\text{-}^d$ antibody had minimal effect on the proliferative response of SEB or GST-I-2. These results indicate that the I-2 response is dependent on MHC II-mediated recognition and, thus, is not a mitogen response.

MHC class II blocking of T cell proliferation was performed as follows. $CD4^+$ T cells (4×10^5) were incubated with 4×10^5 antigen-pulsed antigen-presenting cells as described in section VB above, in the presence and absence of 10 µg/ml monoclonal $anti\text{-}I\text{-}A^b$ or $anti\text{-}I\text{-}A^d$ B (Pharmingen). After 48 hours of incubation, 0.5 µCi $[^3H]$-thymidine was added to each culture for the last 18 hours of the incubation period and collected as before. Results are expressed as mean CPM +/- SD of triplicate cultures.

F. The CD4+ T cell response to I-2 does not require antigen processing

The results set forth above indicate that the I-2 antigen is not a mitogen, but rather either a conventional MHC II processed antigen or a superantigen, which is an antigen characterized, in part, as able to stimulate a proliferative response in the absence of antigen processing. To determine whether the I-2

response requires antigen processing, antigen-presenting cells from normal C57BL/6J mice were fixed with paraformaldehyde to eliminate antigen-processing and then pulsed with GST-I-2 or SEB. As shown in Figure 9A, $CD4^+$ T cells still responded strongly to GST-I-2 and SEB. Furthermore, the response was specific to I-2 since GST alone did not induce a proliferative response. In contrast, fixed antigen-presenting cells pulsed with pigeon cytochrome C (PPC) overnight did not induce PPC specific $CD4^+$ T cells to proliferate (Figure 9B). These results demonstrate that the GST-I-2 proliferative response does not require antigen processing and, therefore, that GST-I-2 acts as a superantigen.

Paraformaldehyde-fixed antigen-presenting cells were prepared and analyzed as follows. The splenic C57 BL/6J $CD4^+$ T cells were cultured with antigen-presenting cells from C57 BL/6J mice that were pulsed with GST-I-2 overnight and for 30 minutes, or fixed with paraformaldehyde for 30 minutes, washed with PBS, incubated with PBS for 30 minutes, and washed twice with PBS. The fixed cells were pulsed with GST-I-2 overnight at 1 µg/ml and 5 µg/ml. GST and SEB were used as the negative and positive control antigens, respectively. As the control for fixation experiment, pigeon cytochrome C (PPC) specific $CD4^+$ T cells were cultured with syngeneic antigen-presenting cells pulsed with PPC overnight, or fixed with paraformaldehyde and then pulsed with PPC overnight. 1 µCi of $[^3H]$TdR was added to the wells for the last 18 hour of a 3 day culture. Results are expressed as mean CPM ± SD of triplicate cultures.

G. TCR Vβ expression is restricted in the responding T cell population

Most superantigens are characterized by a specific TCR Vβ. From a panel of 15 Vβs, Vβ9 and Vβ17 were expanded in $CD4^+$ T cells after GST-I-2 injection. These results confirm that GST-I-2 is a superantigen.

Vβ restriction was characterized as follows. GST-I-2 protein (5 µg) was injected i.v. into normal 8 week old female C57BL/6J mice. One day after injection, spleens were removed and placed into cell suspension. Red blood cells were lysed, and the nucleated spleen cells were resuspended into 5 ml of complete RPMI media containing 5% FCS. Cells (2×10^6) were double stained with PERCP-conjugated anti-$CD4^+$ (Pharmingen) and various FITC-conjugated anti-Vβ (Pharmingen). Stained cells were assessed for $CD4^+$ and Vβ positivity by flow cytometry.

All journal article, reference and patent citations provided above, in parentheses or otherwise, whether previously stated or not, are incorporated herein by reference in their entirety.

Although the invention has been described with reference to the examples above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

We claim:

1. An isolated inflammatory bowel disease associated I-2 polypeptide, comprising substantially the same amino acid sequence as SEQ ID NO: 2.

2. The isolated I-2 polypeptide of claim 1, comprising the amino acid sequence SEQ ID NO: 2.

3. An isolated immunoreactive fragment of an I-2 polypeptide, comprising substantially the same amino acid sequence as a portion of an I-2 polypeptide (SEQ ID NO: 2).

4. The isolated immunoreactive fragment of claim 3, comprising at least ten contiguous amino acids of SEQ ID NO: 2.

5. Substantially purified antibody material that selectively binds an I-2 polypeptide having SEQ ID NO: 2.

6. The substantially purified antibody material of claim 5, which is monoclonal antibody material.

7. An isolated nucleic acid molecule, comprising a nucleic acid sequence encoding substantially the same amino acid sequence as SEQ ID NO: 2.

8. The isolated nucleic acid molecule of claim 7, comprising the nucleic acid sequence SEQ ID NO: 1.

9. A method of diagnosing inflammatory bowel disease (IBD) in a subject, comprising:

(a) obtaining a sample from said subject;

(b) contacting said sample with an I-2 polypeptide, or immunoreactive fragment thereof, under conditions suitable to form a complex of said I-2 polypeptide, or said immunoreactive fragment thereof, and antibody to said I-2 polypeptide; and

(c) detecting the presence or absence of said complex,

wherein the presence of said complex indicates that said subject has IBD.

10. The method of claim 9, wherein the presence or absence of said complex is detected with a detectable secondary antibody that has specificity for a class determining portion of said antibody to said I-2 polypeptide.

11. The method of claim 9, wherein said IBD is Crohn's disease.

12. The method of claim 9, wherein said I-2 polypeptide comprises the sequence SEQ ID NO: 2.

13. A method of inducing tolerance in a patient with IBD, comprising administering an effective dose of I-2 polypeptide, or tolerogenic fragment thereof, to said patient with IBD.

14. The method of claim 13, wherein said IBD is Crohn's disease.

15. The method of claim 13, wherein said I-2 polypeptide comprises the sequence SEQ ID NO: 2.

16. A composition comprising an I-2 polypeptide having substantially the same amino acid sequence as SEQ ID NO: 2, or tolerogenic fragment thereof, combined with a tolerogizing molecule.

17. The composition of claim 16, wherein said I-2 polypeptide has the amino acid sequence SEQ ID NO: 2.

18. The composition of claim 16, which comprises a tolerogenic fragment having at least ten contiguous amino acids of SEQ ID NO: 2.

19. A method of identifying an agent useful in treating inflammatory bowel disease (IBD), comprising:

(a) obtaining a specimen of an enteric bacteria from a patient with IBD;

(b) isolating from said specimen a microbial species that comprises a nucleic acid molecule encoding an I-2 polypeptide;

(c) contacting said microbial species with an agent; and

(d) assaying for reduced growth or viability of said microbial species as compared to the growth or viability in the absence of said agent,

wherein said reduced growth or viability of said microbial species indicates that said agent is an agent useful in treating IBD.

20. The method of claim 19, wherein said IBD is Crohn's disease.

21. The method of claim 19, wherein said agent is an antibiotic.

22. A method of identifying an agent useful in treating inflammatory bowel disease (IBD), comprising:

(a) administering I-2 polypeptide to a non-human animal, whereby one or symptoms of IBD are exhibited;

(b) administering an agent to said non-human animal; and

(c) assaying the level of said one or more symptoms characteristic of IBD,

wherein a reduction in the level of said one or more symptoms as compared to a control level indicates that said agent is an agent useful in treating IBD.

23. The method of claim 22, wherein said IBD is Crohn's disease.

24. The method of claim 22, wherein said I-2 polypeptide has the amino acid sequence SEQ ID NO: 2.

25. The method of claim 22, wherein said non-human animal is a mouse selected from the group consisting of a Gαi2 -/- mouse; a TCRα -/- mouse and a IL-10 -/- mouse.

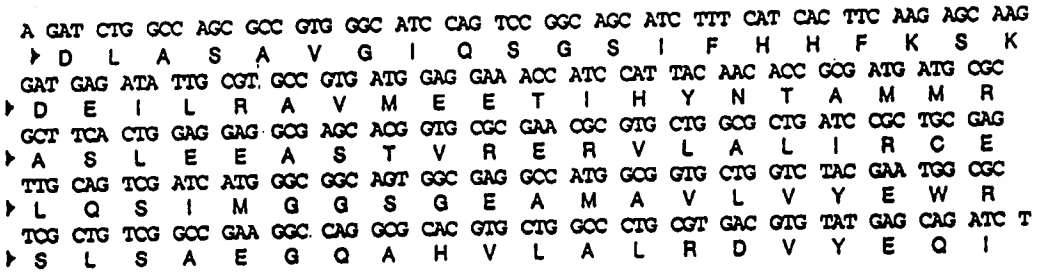


FIGURE 1A

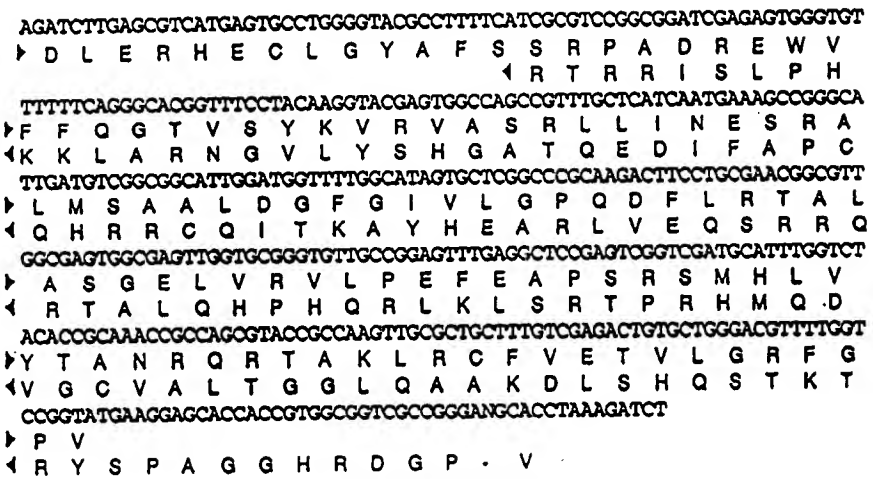


FIGURE 1B

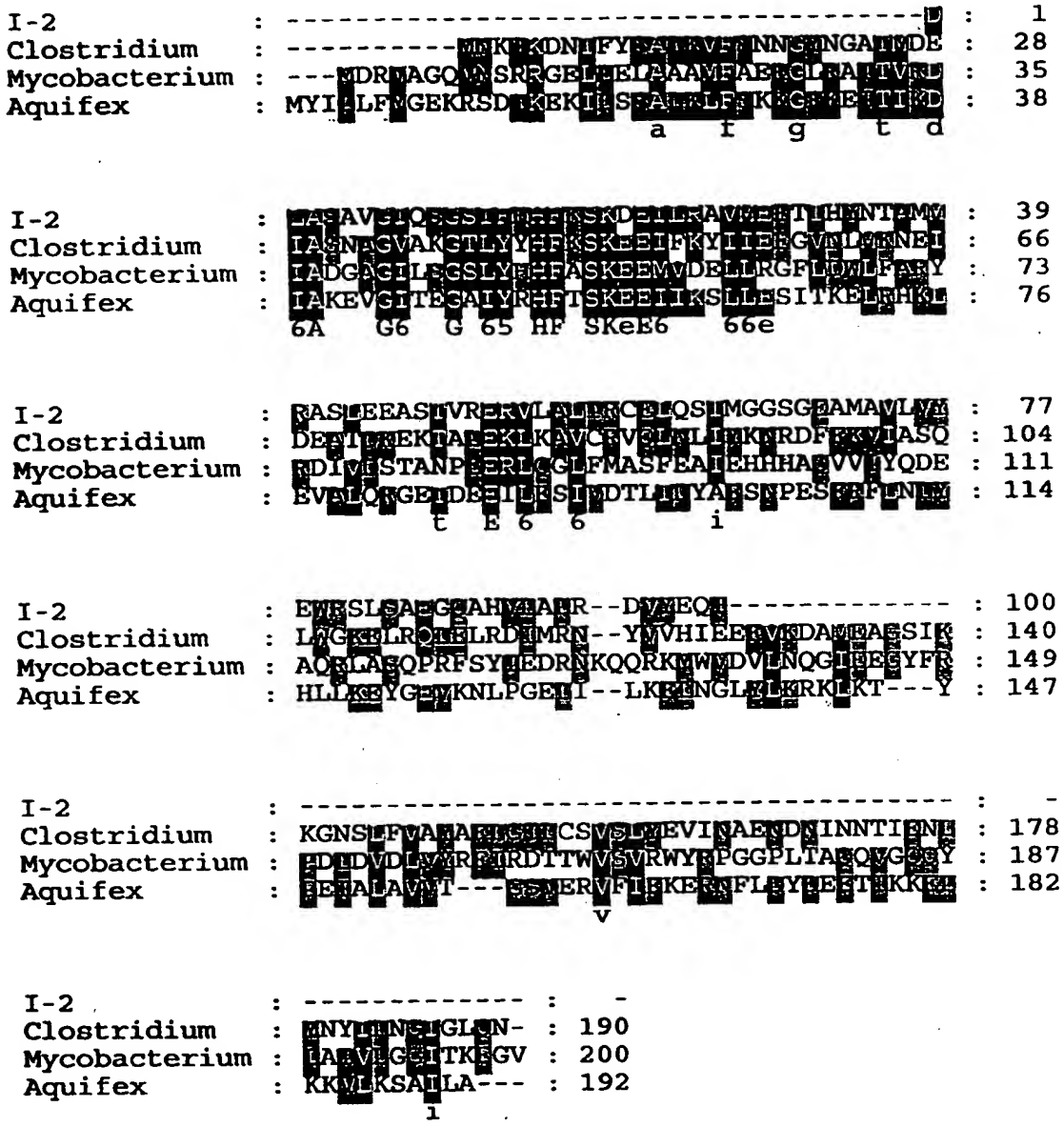

I-2
Clostridium
Mycobacterium
Aquifex
1
28
35
38
39
66
73
76
77
104
111
114
100
140
149
147
178
187
182
190
200
192

FIGURE 2

	Diagnosis	Tissue		N	N(+)	% Pos
	CD	Ileum	Involved	46	25	54.3
	CD	Colon	Involved	35	15	42.9
	CD	Ileum	Uninvolved	14	6	42.9
	CD	Colon	Uninvolved	26	5	19.2
	UC	Colon	Involved	22	2	9.1
	UC	Colon	Uninvolved	20	3	15.0
	Ca	Colon	Uninvolved	15	2	13.3
	Ca	Ileum	Uninvolved	7	3	42.9
	Divertic	Colon	Uninvolved	7	0	0.0
	Append	Colon	Uninvolved	5	0	0.0
	I-Col	Colon	Uninvolved	15	0	0.0
Total				212	61	

FIGURE 3

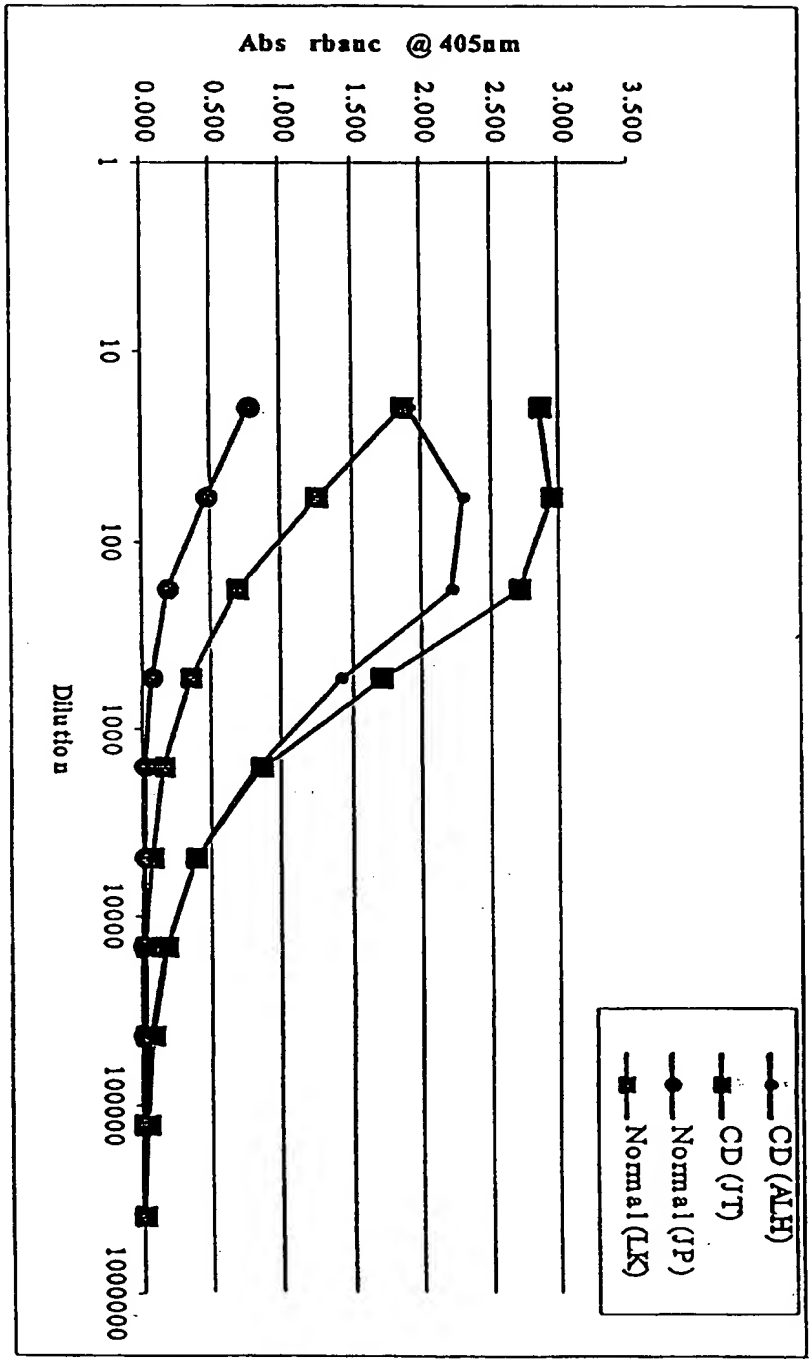

Abs rbanc @ 405nm
0.000
0.500
1.000
1.500
2.000
2.500
3.000
3.500
Dilution
1
10
100
1000
10000
100000
1000000
CD (ALH)
CD (JT)
Normal (JP)
Normal (LK)

FIGURE 4A

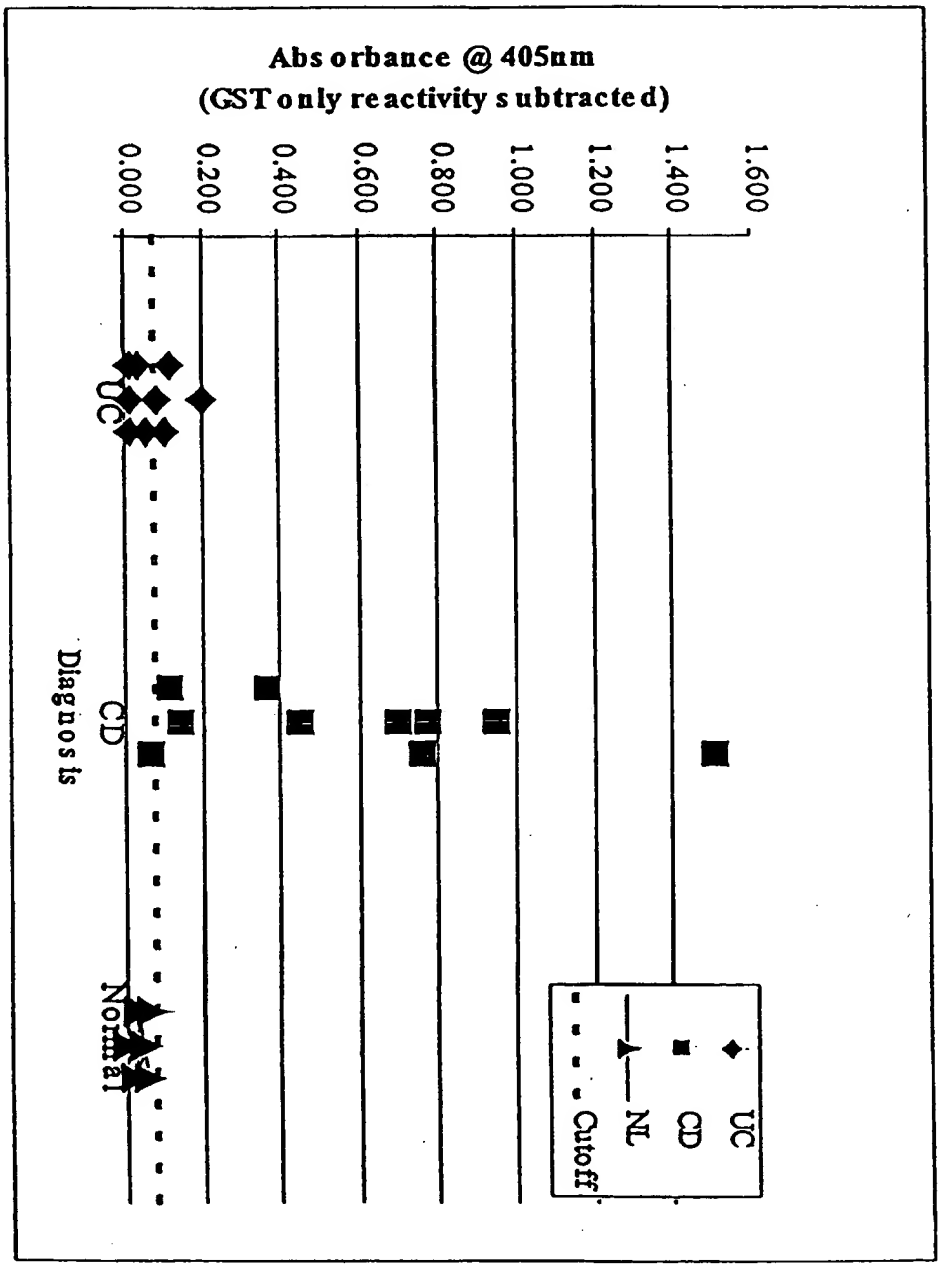

Absorbance @ 405nm
(GST only reactivity subtracted)
0.000
0.200
0.400
0.600
0.800
1.000
1.200
1.400
1.600
UC
CD
Normal
Diagnosis
UC
CD
NL
Cutoff

FIGURE 4B

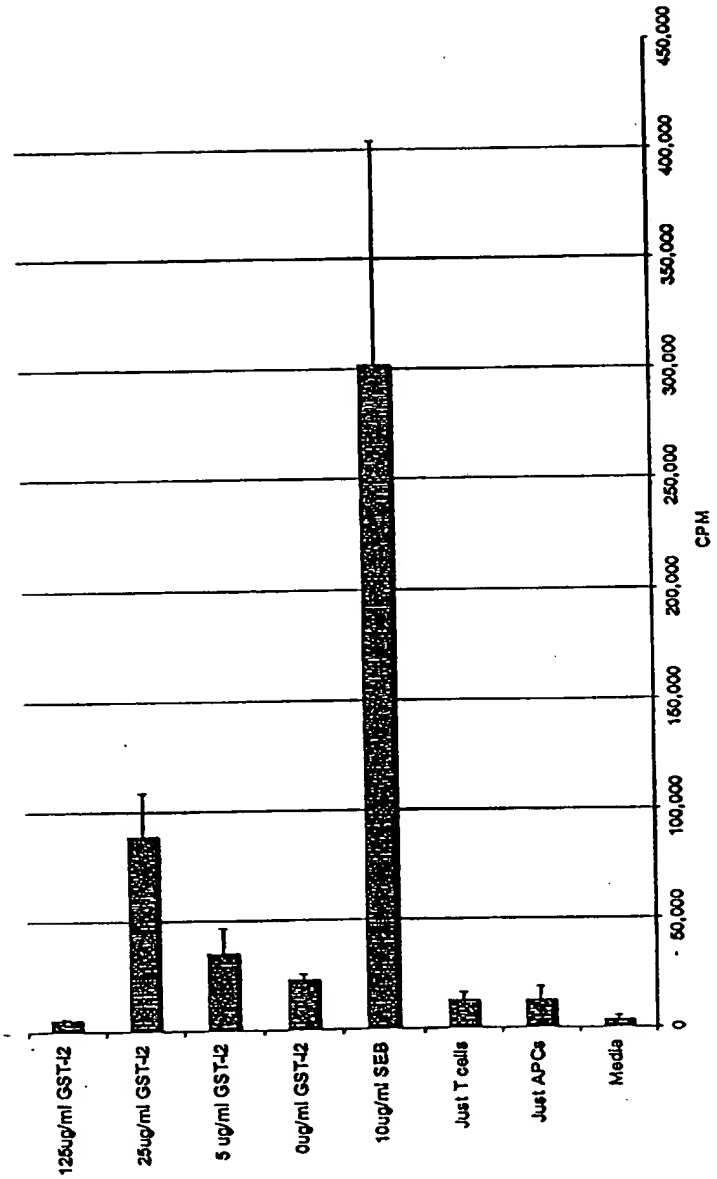

450,000
400,000
350,000
300,000
250,000
200,000
150,000
100,000
50,000
0
CPM
125ug/ml GST-42
25ug/ml GST-42
5 ug/ml GST-42
0ug/ml GST-42
10ug/ml SEB
Just T cells
Just APCs
Media

FIGURE 5

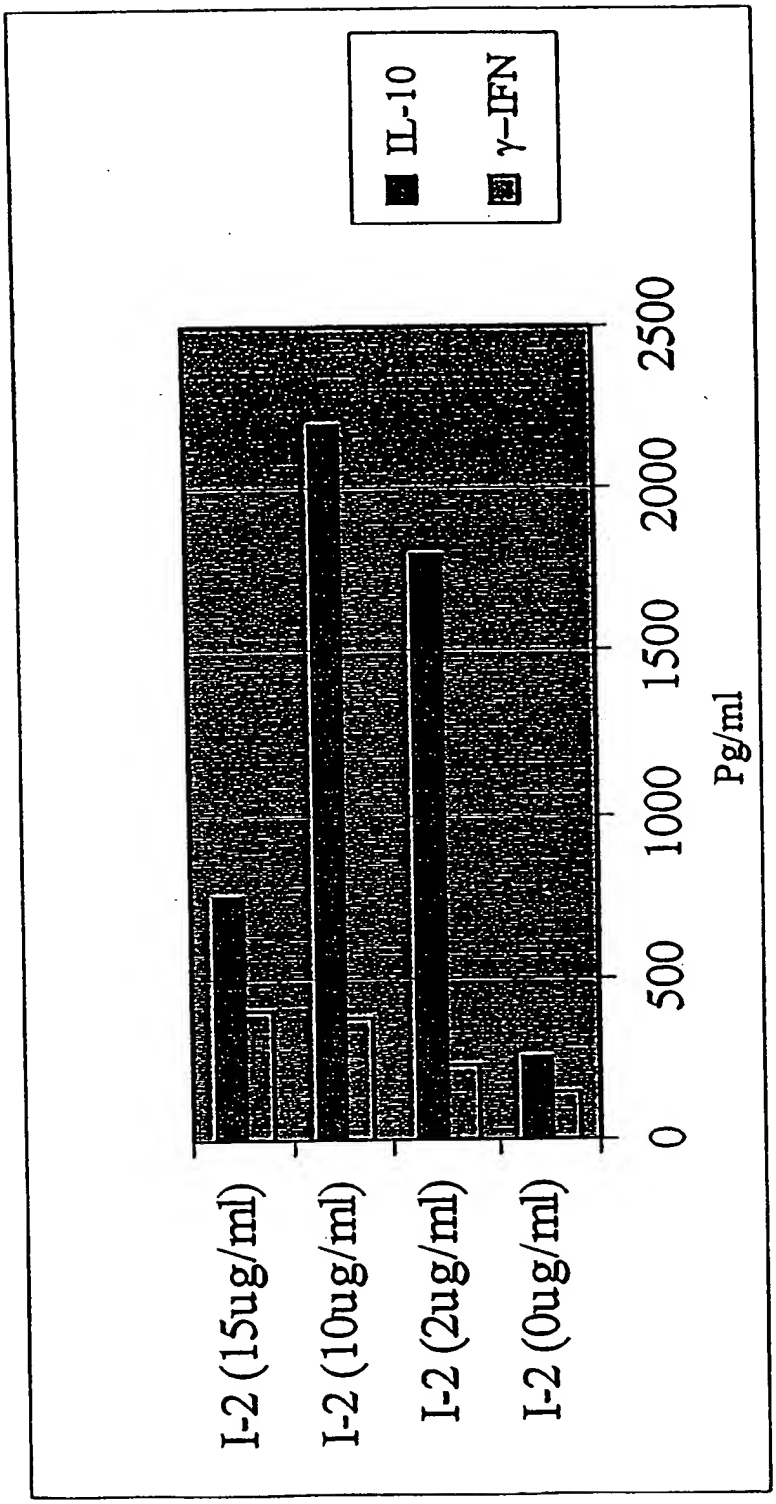

IL-10
γ-IFN
0
500
1000
1500
2000
2500
Pg/ml
I-2 (15ug/ml)
I-2 (10ug/ml)
I-2 (2ug/ml)
I-2 (0ug/ml)

FIGURE 6

Medium	Incubation	Organism
Trypticase soy agar	O_2	Aerobes
McConkey	O_2	*Enterobacteriae*
Sabouraud dextrose with Chloamphenical and gentamycin	O_2	Yeast
Bile eculin agar	O_2	*Enterococcus*
Chocolate	CO_2	*Haemophilus*
CDC	An O_2	Anaerobes
Brucella	An O_2	Anaerobes
EYA+neomycin	An O_2	*Clostridium*
EYA+heat treatment	An O_2	*Clostridium*
CDC+heat treatments	An O_2	*Clostridium*
CCFA+heat treatment	An O_2	*C.difficile/Clostridium*
EYA+ ethanol treatment	An O_2	*Clostridium*
CDC+ ethanol treatment	An O_2	*Clostridium*
CCFA+ethanol treatment	An O_2	*C.difficile/Clostridium*
BBE	An O_2	BFG
LKV	An O_2	Pigmenters
Fusobacterium selective medium	An O_2	*Fusobacterium*
PEA	An O_2	Gpc
CFA	An O_2	*C. difficile*
LAMVAB	An O_2	*Lactobacillus*
RB	An O_2	*Bifidobacterium*
BBE+vancomycin	An O_2	*Bilophila, Sutterella*
Modificed BGSA for Camplyobacter	An O_2, 6%O_2(37C), 6%O_2(42C)	*Camplyobacter*
Campy CVA(CSL)	An O_2, 6%O_2(37C), 6%O_2(42C)	*Camplobacter*
Modified Skirrow	An O_2, 6%O_2(37C), 6%O_2(42C)	*Helicobacter pylori*

FIGURE 7

Figure 8

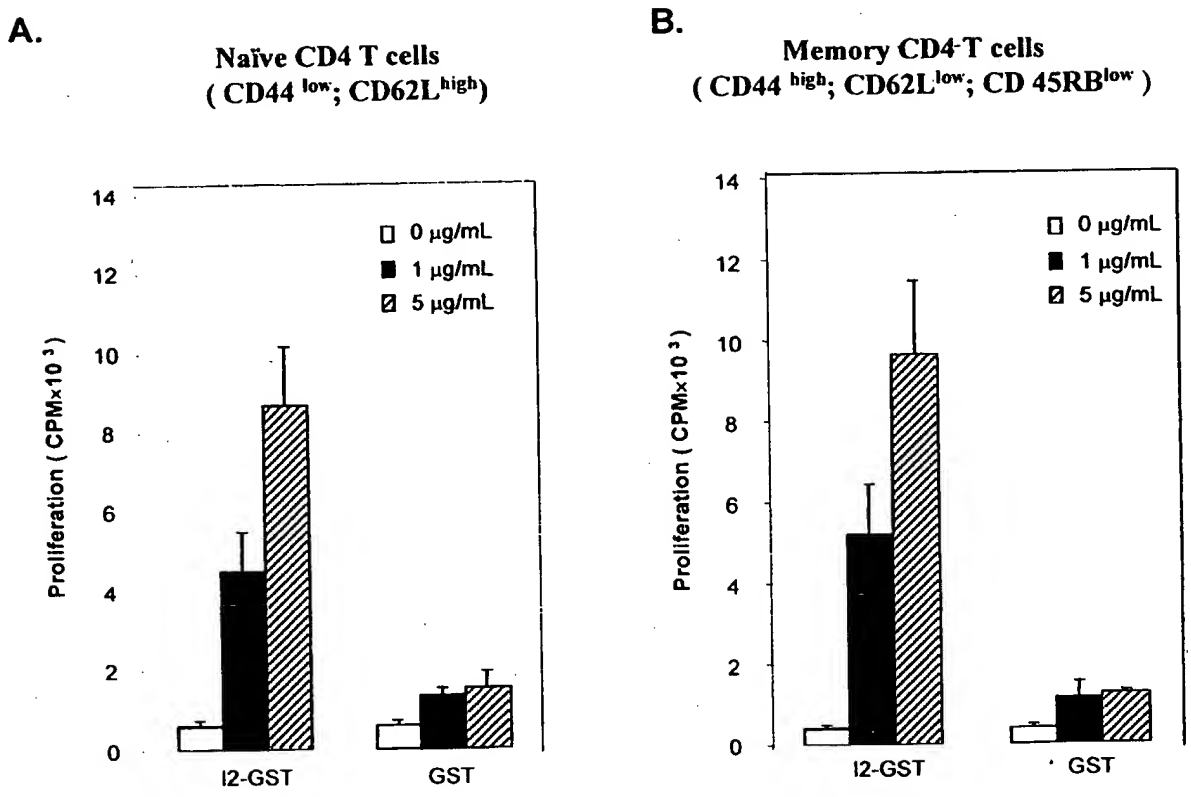
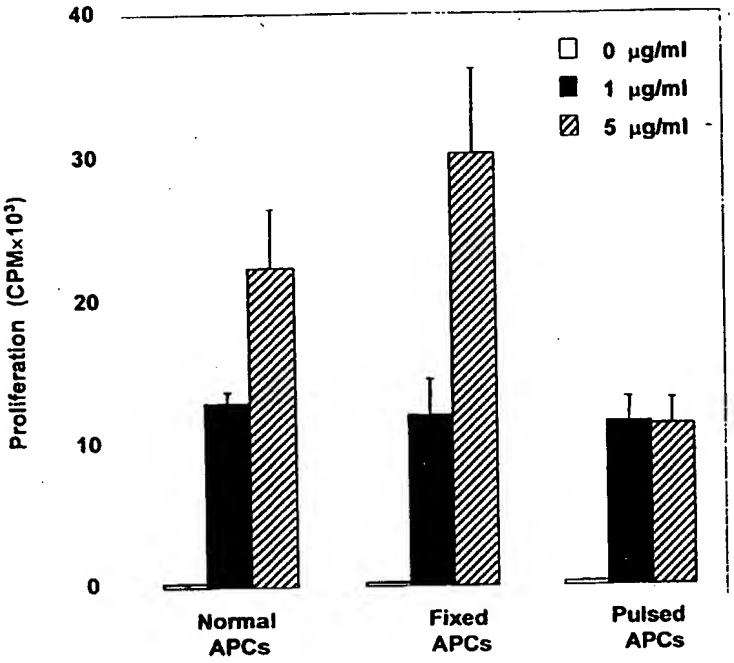

A.
Naïve CD4 T cells
(CD44 low; CD62L high)
Proliferation (CPMx10 3)
0 µg/mL
1 µg/mL
5 µg/mL
I2-GST
GST
B.
Memory CD4 T cells
(CD44 high; CD62L low; CD 45RB low)
Proliferation (CPMx10 3)
0 µg/mL
1 µg/mL
5 µg/mL
I2-GST
GST

Figure 9

A. I2 (splenic CD4+ T cells)



B. PCC (A.E7 cell line)